AVIAN GAIT ANALYSIS

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ABSTRACT

Modern broilers have difficulty walking, and there is ongoing debate as to whether the birds are in pain. Most gait analysis of poultry consists of visual assessment and scoring, and the results are therefore very subjective. In order to describe and quantify gait patterns accurately, a more objective method is required. Only then can differences between the gait of normal chickens and modern broilers be properly described, and attempts made to determine whether the observed gait patterns are the result of the conformation of the birds, or the presence of pain.

Two methods were used in this research. The pedobarograph is a relatively novel method of gait analysis for animals which enables footfall patterns to be recorded, enabling spatial parameters (step length, width and angle) and plantar pressure patterns to be described and measured. A Kistler force plate was then used to measure the three-dimensional ground reaction forces (GRF's) produced during walking. Speed and cadence can be calculated using either system.

Gait patterns are described for normal birds, and for different strains of broilers, raised on different feeding regimes. All the gait parameters were very variable, both between birds, and within the same bird, even when bodyweight and speed were controlled for. Despite the high variability, however, significant differences were identified in many of the gait parameters between the different groups.

The vertical and craniocaudal GRF's of Brown Leghorns showed similar characteristics to those produced in human walking. The peak vertical forces were of a similar order of magnitude in the birds as in humans (125-150 % bodyweight), and the peak craniocaudal forces, and rate of change of force, were closely tied to speed. All the GRF's in the birds increased significantly with increasing speed, except for braking rate (which was more variable) and stance time (which decreased significantly). The mediolateral forces were much greater in the birds than have been reported for other species, however, with peaks of 10-22% bodyweight. Analysis of plantar pressures showed that the pressures were concentrated on the digital pads, with the lowest pressures on the metatarsal pad (<131 kNm^{-2}), and highest pressures on the medial toe (up to 218 kNm^{-2}).

Combined gait analysis and morphometric studies of *ad libitum*-fed selected broilers identified many ways in which their gait deviated from that of relaxed broilers and Brown Leghorns, in ways which would serve to increase stability and decrease stresses on the

skeleton. The *ad libitum*-fed selected birds (compared to restricted-fed strain-mates and relaxed birds of the same weight), had more breast muscle anteriorly, and shorter, wider legs, but with immature bones, of lower % ash content. They moved more slowly, taking short steps, and positioned their feet wide apart and turned 'toe-out' to increase their walking base. They kept their feet on the ground for longer, with short swing periods, and long double contact times. Decreasing the unstable periods of single support, and increasing the stance periods, reduces the peak forces on the skeleton. The slow speed and short steps also reduce the vertical excursions of the centre of gravity. The very wide walking base results in abnormally large mediolateral forces being required to move the centre of gravity over the stance leg, however, increasing the inefficiency of the gait, leading to fatigue. Mediolateral forces in *ad libitum*-fed selected birds averaged 17-21% bodyweight at 6 weeks, compared to 10-11% in the Brown Leghorns, and <5-8% in humans (Biewener, 1992).

Changes in the GRF's were also demonstrated in the slower growing (restricted-fed) broilers with age. In these birds, both the peak vertical forces and the peak mediolateral forces decreased with age, despite the fact that the birds increased in size (and girth) and their speed of movement remained similar. This indicates that the birds developed gait optimisations as they grew (as is the case with children), possibly because the slower growth allowed the body to develop more in proportion.

Analysis of the gait of a small number of lame birds demonstrated marked differences in the spatial and force parameters between the sound and lame limb. The peak forces on the lame leg were dramatically reduced, and compensatory increases were seen in the GRF's of the sound limb. The birds used several methods to avoid fully loading the lame leg: flapping the wings to raise the centre of gravity, pushing up on the sound limb prior to placing the lame leg down, and flexing the lame limb as it starts to weightbear.

There were no changes in the GRF's or temporal parameters of gait of broilers given analgesic (carprofen). Unfortunately, this does not prove conclusively that the birds were not in pain, as analgesic efficacy has not been validated in broilers, and it is also possible that the parameters under test do not change in the presence of pain. However the incidental finding of old blood in synovial fluid samples of over 50% of the *ad libitum*-fed broilers adds to the welfare concerns. A second interesting finding was the high alkalinity of the fluid (median pH 8.15 - 8.40), with the possible consequences on intra-articular pharmacodynamics. Although the analgesic study proved inconclusive, results of the gait and morphometric studies demonstrate that it is possible to explain the gait patterns of *ad libitum*-fed selected broilers based on their body conformation alone.

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DECLARATION

I declare that the work contained in this thesis is my own, and has not been submitted for any previous degree. Reference to the work of other authors has been duly acknowledged, as has any assistance given to me during the course of this research.

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ABBREVIATIONS

dct = double contact time

CG = centre of gravity

CP = centre of pressure

CM = centre of mass

GRF = ground reaction force

Ek = kinetic energy

Ep = potential energy

u = dimensionless speed

FBD = Franks, Betts and Duckworth (authors of a series of papers on the pedobarograph).

tmt = tarsometatarsus / tarsometatarsal

tt = tibiotarsus / tibiotarsal

CrCd = craniocaudal

lat = lateral

Fz max – 100 = peak vertical force minus 100% of bodyweight

Fz slope = rate of change of vertical force at the beginning of stance phase.

Fz max = peak vertical force

Fy max = peak propulsive force

Fy min = peak braking force

Y ratio = ratio of Fy max / Fy min

Fx max and **Fx min** (over a run) = peak forces in the mediolateral plane.

Fx max (over a step) = peak mediolateral propulsive force

Fx min (over a step) = peak mediolateral braking force

X ratio = ratio of Fx max / Fx min

Brake % = % of a single stance period spent braking (and therefore equal to 100-% of single stance time spent in propulsion).

Braking rate = rate of change of craniocaudal braking force in one stance period

Propulsion rate = rate of change of craniocaudal propulsive force in one stance period

Braking integral = area under force-time curve for braking period of one stance period (also known as the braking 'impulse')

Propulsion integral = area under force-time curve for propulsion period of one stance period also known as the propulsion 'impulse').

CHAPTER 1:

GENERAL INTRODUCTION

The high level of musculoskeletal disorders seen in modern broilers (meat production birds), causes both economic and welfare concerns. While the economic losses arising from mortality and carcass condemnation are quantifiable, the welfare problem is less easy to define. Much work has been done on the pathophysiology of the various musculoskeletal disorders, but little is known about the biomechanical effects or painful consequences of the pathologies. The abnormal gait of many of the birds may result from pain, or from biomechanical problems. The latter may not be painful, but may still compromise the bird's welfare if, for example, it is unable to reach the feeders.

Large-scale gait analysis of poultry is carried out using the Bristol Gait Scoring System (Kestin *et al*, 1992), a method which involves visual assessment and the allocation of a subjective gait 'score'. While such a subjective method is easy to apply, it has obvious limitations in terms of reliability and repeatability between observers. The aim of the work presented in this thesis was to develop and test a more objective method of gait analysis to use with poultry. Such a system could then be used to describe and quantify various gait parameters in broilers, and compare them with those of birds with 'normal' locomotor function. Having quantified the various gait parameters, the involvement of pain in the gait pattern could then be investigated with the use of analgesics. It is also possible that the heavy selection for rapid growth rates and increased meat production has produced a bird whose conformation alone makes normal gait difficult. Thus it is essential to conduct a more detailed examination of the biomechanics of walking, and the scaling differences (in body conformation and proportions) between the modern broiler and its predecessors.

1.1 LAMENESS AND THE POULTRY INDUSTRY

In 1992, a damning report on the welfare of broiler chickens was produced by the Farm Animal Welfare Council (FAWC), following a survey commissioned by the Ministry of Agriculture Fisheries and Food (FAWC, 1992). The report stated that the level of leg problems in broilers was unacceptable, and made it the responsibility of the Industry to ensure a 'significant reduction' in the 'numbers and severity' of leg problems prior to re-assessment in 5 years. Although the FAWC report did not contain figures, a UK survey by Kestin *et al* (1992), estimated that 90% of intensively reared, commercial broilers had a detectable gait abnormality, and 26% were severely lame. A survey of the American broiler industry found a mortality rate of 1.1%, with a further 2.1% of carcasses being downgraded

due to leg problems, at an estimated annual cost of $80 to $120 million (Morris, 1993, quoted by Hester, 1994).

Recent figures are difficult to obtain, although a review of skeletal disorders (Thorp, 1994), quoted mortality rates of between 2 and 8 % in growing broilers as a result of skeletal diseases, and estimated that approximately 30% of hens had bone fractures at the end of lay. Mortality from similar causes in growing turkeys was quoted as being 3 - 4%. In 1995, approximately 19.5% of 6 million carcass condemnations were attributed to leg problems alone (Yogaratnam, 1995).

Lameness can be defined as a disability of the legs or feet, and is usually apparent as an abnormality of gait, that is, the manner of walking or running (Collins, 1993). Many good reviews of musculoskeletal disorders in poultry are available, most of which group the problems into those of infectious or non-infectious origin (Riddell, 1992; Thorp, 1996). The commonest infectious causes of lameness include chondritis and osteomyelitis resulting in proximal femoral degeneration (*Staphyloccocus aureus and Escherichia coli*), synovitis (*Staphyloccocus aureus* and *Mycoplasma synoviae*), and foot pad dermatitis (*Staphyloccocus aureus*) (Hester, 1994; Thorp, 1994; Ekstrand *et al*, 1998; McNamee *et al*, 1998). Less commonly, viral infections can cause lameness, for example sciatic nerve demyelination in Marek's disease, and viral arthritis and tendonitis from reovirus infection (Hester, 1994).

Of the non-infectious causes, most are related to the production stresses placed on the modern, highly selected, rapidly growing broiler. These can be divided into two main groups based on the age and type of bird affected (Thorp, 1996):

Young, rapidly growing broiler chicks:

Most disorders seen in this age of bird involve disturbances to the growth plates, resulting in bone deformities, e.g. torsional and angular deformities. The incidence varies widely, but has been estimated at 0.5 to 25% (Julian, 1984). The most common deformity in broilers is valgus angulation of the intertarsal joint, with lateral twisting of the distal tibiotarsus (Duff and Thorp, 1985b). A greater frequency of deformity has been found in the right limb, suggesting that limb dominance may exist in broilers (Duff, and Thorp, 1985a, Duff, 1986). Although small degrees of intertarsal valgus may be considered normal, more than 20 degrees is though to be abnormal, as is any varus deformity (Duff and Thorp, 1985a, 1985b). Various causes have been suggested, including altered load bearing and functional activity, and rapid growth rates (Duff, 1986; Riddell, 1992; Hester, 1994; Thorp,

1996). Other studies implicating delayed cortical bone formation, increased metaphyseal modelling, decreased plasma 1,25 dihydroxycholecalciferol and prostaglandin levels, and concurrent infectious or metabolic disease, are quoted in Thorp (1994).

Other major problems include the development of abnormal cartilage (e.g. in dyschondroplasia), and disturbances of bone formation (osteodystrophy), although the two are often linked (Thorp, 1994). Tibial dyschondroplasia (TD) deserves special mention due to the high incidence seen in some broiler flocks: McNamee *et al* (1998) quotes studies by Prasad *et al*, (1972) and Riddell (1992) which found levels as high as 30% in flocks. A typical TD lesion appears as an irregular mass of avascular and sometimes necrotic cartilage below the epiphyseal plate of the proximal tibiotarsus, extending into the metaphyseal region (Leach & Nesheim, 1965; Poulos *et al*, 1978). Despite the fact that the incidence and severity can be reduced by genetic selection and husbandry (Leach and Nesheim, 1965; Sheridan *et al*, 1978; Thorp, 1996), a recent study found gross TD lesions in 25% of broilers in 2 commercial flocks (McNamee *et al*, 1998)

Adolescent and adult breeding stock of meat type:

These are mainly affected by degenerative disorders, particularly of the cartilage of the hip, stifle and intertarsal joints (Thorp, 1994). A *post-mortem* survey of male broiler breeders found degenerative changes in the articular cartilage of 46% of the birds (Duff and Hocking, 1986), while a survey of male breeding turkeys found degenerative changes in the hip joints of 90% of the birds (Duff *et al*, 1988). More intensive genetic selection and heavier weights in the males result in a higher incidence of musculoskeletal disease than in females (Hocking, 1992).

A high incidence of partial or total ligament or tendon rupture is also reported in adult broiler breeding fowl. Eighty-five percent of birds in the Duff and Hocking study (1986) had partial or total rupture of tendons or ligaments (most commonly in the ligaments of the knee and tarsal joints), and rupture of the gastrocnemius and flexor tendons is also common (Duff and Randall, 1986).

Little is known about the painful consequences of the various types of lameness described above. Lameness can be the result of pain arising from the limb making the bird reluctant to use the leg (Gentle and Corr, 1995), or from anatomical changes, which could alter mechanical function without necessarily being painful. While it is likely that lameness resulting from trauma or infection is painful, lameness due to mild angular deformities or TD may simply be biomechanical. Lynch *et al* (1992), for example, found that only 50% of

birds with radiological evidence of TD lesions were lame, while McNamee *et al* (1998), found gross lesions suggestive of TD in 11.4% of lame birds, and 13.6% of sound birds.

1. 2 THE ROLE OF PAIN IN LAMENESS

A number of studies have attempted to assess the role of pain in lameness arising from different types of musculoskeletal pathology in birds. The International Association for the Study of Pain (1979) defines pain as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage'. We should therefore start by asking if birds are capable of feeling pain as we understand it, as care must be taken in ascribing subjective feelings and emotions to animals. Possession of the necessary neural pathways connecting peripheral nociceptors to the higher brain centres analogous to the human cerebral cortex constitutes one of the criteria proposed by Bateson (1991), for pain perception in animals. A review by Gentle (1993) describes the various types of peripheral nociceptors found in avian tissues. These include mechanical, mechano-thermal, thermal and articular receptors, all of which relay information, via the dorsal horn of the spinal cord, to the medullary reticular formation and thalamus. He describes how nociceptive stimulation results in either escape responses or crouching by the bird, the immobility depending on the stimulus. This meets the definition of pain given by Zimmerman (1986) which states that pain in animals is ' An aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance, and may modify species specific behaviour, including social behaviour'.

Attempts have been made to investigate pain by testing the birds' responses to anti-inflammatory and analgesic drugs, with variable results. Early work using morphine produced variable effects, low doses sometimes producing hyperalgesia (Bardo and Hughes, 1978; Fan *et al*, 1981; Hughes, 1990). A study of adult male turkeys with degenerative hip disorders showed increased spontaneous activity in the birds following injections of the anti-inflammatory steroid betamethasone, causing the authors to suggest that the birds were in a state of chronic pain (Duncan *et al*, 1991). In contrast, a later study on male broiler breeders with leg 'disorders' showed a decrease in walking speed following betamethasone administration (Hocking, 1994). Similar effects were also seen following the administration of naloxone, causing Hocking to suggest that endogenous opioids may produce analgesia in these birds. Endogenous opioids are the body's 'natural' analgesic drugs, which can be produced in stressful situations (known as 'stress-induced analgesia').

This phenomenon has been clearly demonstrated in birds with unilateral urate arthritis, where changes in attention initiated by the birds being placed in a novel environment suppressed the pain-related behaviours normally produced by urate arthritis (Gentle and Corr, 1995). A similar decrease in pain-related behaviour (one-legged standing, resting in a hypoaesthetic state) in birds with unilateral urate arthritis, was seen following intra-articular administration of bupivicaine (Hocking *et al*, 1997). Most recently a study found that lame broilers chose to eat greater quantities of food containing carprofen (a non-steroidal anti-inflammatory drug with analgesic properties), than non-lame birds (Pickup *et al*, 1997). This demonstration of 'self-medication' suggests that the birds were feeling pain, however the subjective scoring method (Kestin *et al*, 1992) used to ascribe the degrees of lameness and assess the response weakens the experiment.

Although these studies suggest that certain locomotor disorders may be painful, this has not been proven conclusively, due to the variable effects of certain analgesic drugs in birds, and more importantly, the subjectiveness of the methods used to assess the abnormal locomotion and describe the responses to treatment. If we consider clinical decision making in cases of gait abnormality in humans, there are distinct stages, which are described by Gage (1983) and Rose (1983):

a) assessment (history and physical examination) followed by gait analysis,
b) hypothesis formation (possible causes of the observed abnormality),
c) hypothesis testing (by using a different method of measurement, or by attempting to modify the gait in some way e.g. by using local anaesthetic to desensitise an area).

There have been no systematic attempts to apply a similar protocol to the study of avian lameness. As mentioned previously, most assessment of lameness in poultry is presently done using the 'Bristol Gait Scoring System', developed by Kestin *et al* (1992): a visual assessment is made of the birds gait and a subjective gait "score" allocated to each bird, based on a scale of 0 to 5. On the basis of this observational study, Kestin suggests that the birds' welfare becomes compromised between gait scores 2 and 3, and that there is likely to be chronic pain and discomfort associated with the immobility of birds with gait scores of 3, 4, or 5. There are a number of obvious drawbacks to using such a subjective, visual method of assessment. It is transitory, giving no permanent record; it allows only the observation of movements, not forces; and is dependent on the skill and experience of individual observers (Whittle, 1991). Events taking less than one twelfth of a second cannot be perceived by the human eye, and it is difficult to observe several simultaneous events

(Gage and Õunpuu, 1989). The reproducibility of visual gait analysis has been shown to be only moderately reliable by Krebs *et al* (1985).

A subjective system simply makes an assessment of the gait, rather than an analysis (any sort of measurement), and completely ignores hypothesis formation and testing. It is also important to realise, particularly when investigating lameness, that the observed gait pattern is not only a result of the pathology, but also of the subjects' attempts to compensate for it (Rose, 1983). This important point is reinforced by Whittle (1991), who comments that other factors must also be considered, such as the possibility that a pathological process may include an element of variability e.g. ataxia. Simply describing the way the bird is walking gives little information about these underlying processes.

1. 3 BIOMECHANICS OF LOCOMOTION

The forces that organisms generate and withstand as they function mechanically, the effects of these forces on the structural elements of the body, and the way in which the material properties relate to the functional requirements are studied in the field of biomechanics (Biewener, 1992).

The main force acting on the body is the ground reaction force (GRF), which acts on the foot, and can be resolved into three orthogonal forces: mediolateral (Fx), craniocaudal (Fy, the 'braking and propulsive force'), and vertical (Fz). These have been well established in man (Winter, 1990; Whittle, 1991), horses (Goodship *et al*, 1982; Merkens *et al*, 1988) and dogs (Budsberg *et al*, 1987; DeCamp, 1997). Shear, rotation and twisting forces are considerably more difficult to measure. The largest of the three orthogonal forces is the vertical force: when a subject is static, the total downwards force will be the sum of all the segment masses x gravity, which equals the bodyweight (Currey, 1975; Winter, 1990).

The 'centre of gravity' (CG) is an important concept in biomechanics, which describes the point at which the entire weight of the body is concentrated. In standing humans, the CG is estimated to be at a point approximately 55% of the height, just anterior to the second sacral vertebra (Saunders *et al*, 1953). The CG is therefore distinct from the 'centre of mass' (CM) - it is basically the net location of the CM in the vertical direction only, as defined by gravity (Whittle, 1991). A third important concept is the 'centre of pressure' (CP), which can be thought of as being ' the neuromuscular response to imbalances in the body's centre of gravity': the internal joint forces and moments generated by muscle, ligament and bony structures balancing the externally applied forces (Winter,

1990). Thus the CP is the point through which a single resultant force appears to act (Whittle, 1991).

Most of the biomechanics of locomotion are involved with supporting the CG, and maintaining stability while propelling it forwards without undue vertical or lateral movement. Perhaps the most basic principle is that a subject will remain stable as long as a line of force passing vertically down from the CG remains within the base of support provided by the limbs (Whittle, 1991; Roggero *et al,* 1993). The ways in which the body achieves this during locomotion will be expanded upon in the section on the gait cycle (1.5). In outline, the foot exerts a forward force in the first half of its period of ground contact during walking (as it brakes), followed by a backward force in the second half (as it pushes off), all the while being subject to the vertical force created by the body weight (Jacobson and Hollyday, 1982; Whittle, 1991). Lateral forces are usually relatively small when walking in a straight line (Alexander, 1977).

The forces acting on the body create stresses in the structural elements in a number of different ways. If the stresses are too great, the skeleton will fail, and so it needs to develop during the growth of the animal in a way that keeps the forces within 'safe limits' (Biewener *et al,* 1986; Frost, 1997; Skerry, 1997). The animal in theory has two 'choices': it can accept a poorer locomotory performance or safety factor in its skeleton (a poor adaptation, and therefore unlikely to be incorporated into the genotype), or it can change shape (of the whole body, or skeletal components) and / or increase the mechanical efficiency of the structural material itself (Currey, 1975). Changes in body shape and scaling of animals has been studied extensively (particularly by Alexander), and will be discussed in detail in the next section. The alternative, that of increasing the mechanical efficiency of the structural material, can be achieved by either improving the quantity or the quality of the material. Although an increase in bone mass will improve stiffness and resistance to bending, it brings the obvious disadvantages of increased weight, increased moment of inertia, increased metabolic cost etc (Currey, 1975). An improvement in the 'quality' of bone however, by increasing its mineral content, results in a dramatic increase in maximum and yield stress, and modulus of elasticity, and therefore its ability to resist the demands placed upon it (Currey, 1975, Currey, 1988). This was demonstrated in a study which showed that although female broilers had thinner skeletons than the males, the higher mineral content of bone in the females effectively counteracted this in terms of bone stiffness, by altering the elastic components of the bone (Rose *et al,* 1996).

Maintenance of bone quality during life is dependent upon the forces produced by loads applied to the skeleton (as stresses), which create strains within the bone. Many studies have looked at the effects of different loads on bone mass and density, and both the magnitude and direction of the load is known to be important (Jones *et al*, 1977; Biewener *et al*, 1986; Van der Mulen *et al*, 1993; Frost 1994a, 1994b; Fisher *et al*, 1996). A study using different patterns of loading confirmed that in the absence of load, bone mineral content (bmc) declines, as does the cross-sectional area of the bone, whereas loading increases both bmc and bone deposition, primarily on the periosteal surface (Rubin and Lanyon, 1987). These authors also showed that loading had to be dynamic: static loading had little influence on maintaining the balance of the skeleton, producing no increase in new bone formation. The low levels of activity seen in modern poultry are unlikely to reproduce optimal loading conditions. A study comparing trabecular bone volume in birds kept in different housing conditions, for example, demonstrated that male birds kept on the floor had significantly greater bone volume than caged males (Wilson *et al*, 1992). More recently, a similar study showed that after only 20 days, birds moved from battery cages to floor pens had stronger bones (as assessed by increased breaking strength and cross-sectional area), and increased ash values (Newman and Leeson, 1998).

In the long bones, the predominant stresses are produced by the muscles as they work against bodyweight and gravity, and so all parts of the bone cortex are subject to significant strains at some point during normal activity (Van der Mulen *et al*, 1993; Frost, 1997). It is thought that each region of the bone has a genetically determined 'optimal strain environment' in terms of magnitude and pattern of loading, and any deviation from this induces bone remodelling (Biewener *et al*, 1986; Rubin and Lanyon, 1987). Various stress thresholds for different modelling activities have been described in terms of microstrains by Frost, where 1000 microstrain in compression would shorten a bone by 0.1% of its original length (Frost 1994a, 1994b, 1997). He suggests that 1000 microstrain is the set point of the normal modelling range, with 3000 microstrain being the set point of the microdamage range (below which damage resulting from normal strain/destrain is repaired by basic multicellular units), and 25000 microstrain is the bones ultimate strength, above which it fails. Thus a basic prerequisite for a healthy skeleton is that the bones should be dynamically loaded, to produce sufficient strain to maintain bone density and mass, but within 'safe levels' i.e. below those levels which induce excessive microdamage or failure. This theory is described in detail in relation to joints in an excellent paper by Frost (1994a), with a follow-up paper describing how arthropathies develop when the 'safe limits' are exceeded (Frost, 1994b).

Within the skeletal system, energy absorption is inversely proportional to stiffness, and while stiffness is required to resist the loads produced in normal locomotion, the skeleton must also be able to absorb energy without breaking. As well as the mechanical properties of the bone itself, several adaptations aid in this. Many bones lie buried deep in soft tissues which protect them from direct injury, and they also have 'shock-absorbing' mechanisms such as cartilage, to lengthen the duration of impact, and so avoid high local stresses e.g. at joints (Currey, 1975; Frost 1994a, 1994b). These mechanisms for decreasing impact forces are relatively minor, however, compared to the benefits of changing shape e.g. by flexing the joints (Collins and Whittle, 1989).

Thus the biomechanics of locomotion are complex, and it is likely that the modern broiler, with its rapid growth and heavy bodyweight, will be subject to different biomechanical stresses than its predecessors. Although direct measurements of bone strains will not be made in this thesis, inferences can be made from the forces created during walking in the birds, in combination with various morphometric measurements made during growth and at *post-mortem* examination. Because of selection for increased breast muscle, the conformation of the broiler has also changed, and so it is important to consider scaling effects and how they might affect locomotion.

1. 4 SCALING EFFECTS

When differently sized structures retain the same shape i.e. are geometrically similar, they are said to scale with isometry. In such cases, all linear dimensions scale proportional to (α) body volume (v) $^{1/3}$, equivalent to mass (m) $^{1/3}$ in structures of equal density. As cross-sectional area scales α (length) ^{2}, and volume to (length) ^{3}, it is obvious that increases in length produce a much greater increase in body volume than cross-sectional area. If cross-sectional area is considered as the support base, it can be seen that to keep the ratio of cross-sectional area per unit volume the same, the object must change in shape (become wider) as it increases in size (Maynard Smith, 1968; McMahon, 1973; Swartz and Biewener, 1992).

When there is a disproportionate change in shape with overall size, organisms are said to scale allometrically, in either a positive or negative way, with respect to isometry (Schmidt-Neilsen, 1975; Swartz and Biewener, 1992). This may allow certain functional capabilities to remain constant at different sizes. It is possible to determine whether a particular structure shows isometric or allometric scaling by making various measurements

(length, diameter and cross-section), and performing linear regression analysis of the log-transformed data (Swartz and Biewener, 1992). Body mass is a suitable measure of overall size, but care must be taken to control for seasonal and nutritional fluctuations of body mass, and the effects of sexual dimorphism (Swartz and Biewener, 1992; Rose *et al*, 1996).

The modern broiler has been intensively selected for rapid growth rates and increased breast muscle mass. A comparison of studies over the last 20 years shows that bodyweight in commercial broilers has almost doubled at 56 days (Lilburn, 1994). Breast muscle mass, however, reaches 101.5g by 4 weeks (Acar *et al*, 1993), compared to 57.3g at 9 weeks in 1970 (Lilburn, 1994 quoting Halvorson and Jacobson, 1970). Thus over 20 years of selective breeding, the bodyweight of broilers has almost doubled, while the breast muscle mass is almost twice as great by half the age. Unfortunately for the birds, the amount of leg bone has not increased in proportion (Lilburn, 1994), which is not surprising, considering the high genetic correlation between bodyweight and muscle mass, and the low correlation between body weight and skeletal elements (Emmerson *et al*, 1991). If the cross-sectional area of the bones available to resist mechanical loads does not vary in proportion to those loads, the stresses on particular skeletal elements may exceed 'safe' limits (McMahon, 1973; Swartz and Biewener, 1992). As force (F) normally varies in proportion to weight (W) i.e. $F \alpha W^{1.0}$, increases in bodyweight will result in increases in force greater than the increases in cross-sectional area (as area varies in proportion with $W^{2/3}$). As stress = force / area, if the shapes of the support elements do not change, stress will be increased with increasing size $\alpha W^{1/3}$ creating the risk of mechanical failure (Swartz and Biewener, 1992). Thus larger animals would have to alter aspects of their mechanical function to keep forces below critical levels. It is possible that the locomotor ability of modern broilers may be compromised in this way if their conformation has not 'scaled' appropriately to their increased weight.

An alternative scaling theory was proposed by McMahon (1973, 1975), who suggested that geometric similarity was unlikely, and instead the aim could be to maintain elastic similarity i.e. normalising the elastic deflection of the structure under its own static weight. For this to happen, length (l) would have to increase as the 2/3 power of diameter (d) - as opposed to geometrically similar animals, where lengths and diameters of corresponding parts are proportional to (body mass) $^{0.33}$. He describes a model where, if $W \alpha ld^2$, then (l^3 being proportional to d^2), length $\alpha W^{1/4}$ and diameters $\alpha W^{3/8}$. This

implies that bone length is proportional to (bone diameter) $^{0.67}$ (Alexander *et al*, 1979). McMahon quotes other studies to support his hypothesis (Brody, 1945 on cattle; Stahl and Gummerson, 1967 on primates). The theory was tested on a wide variety of animals by Alexander *et al* (1979), however, and found to only really apply to Bovidae, prompting the suggestion that elastic similarity may be maintained within a family, but not throughout higher taxa.

One of the first studies to consider postnatal ontogeny of the muscular and skeletal systems in the context of each other was carried out on rabbits by Carrier (1983). In theory, the load required to break a bone should be proportional to the forces imposed on the bone by the muscles, which will scale as the cross-sectional area of the muscles (Hellam and Podolsky, 1969). In geometrically similar animals, muscular force scales as an area, so the breaking load would scale as (body mass) $^{0.667}$. To maintain locomotor function with increases in body size however, muscular force needs to scale isometrically with body mass, causing breaking load to be proportional to (body mass) $^{1.0}$. This was found to be the approximate case with breaking strength of the 3rd metatarsal scaling to (body mass) $^{0.986}$, however that of the femur scaled to (body mass) $^{0.749}$, a difference which Carrier could not explain.

It is clear that the relationships between body size, muscular force and bone strength are complex. It is important however to examine scaling in the modern broiler to determine the possible consequences of the increased bodyweight and breast muscle mass on the musculoskeletal system, and locomotor ability of the birds.

1. 5 THE GAIT CYCLE

General description:

Gait has been described as 'a given pattern of footfalls' (McGee, 1968), and a gait cycle defined as the period 'between two successive occurrences of one of the repetitive events of walking' (Whittle, 1991). In its simplest form, each foot has a stance phase (when it is in ground contact), and a swing phase (when it is lifted off the ground and advanced forwards). There can also be periods of double support (when both feet are on the ground), and floating phases (when no feet are on the ground during running or hopping). Beyond this, the terminology associated with gait is sometimes confusing, as the naming of various parts of the cycle is not consistent between studies. In this thesis, the terminology favoured by Whittle (1991) will be used, and is listed in the Glossary.

In general, gait patterns are either symmetrical, where the feet of a pair move alternately, half a cycle out of phase with each other (e.g. normal human walking), or asymmetrical (such as used by a galloping horse), where the footfalls of a pair of feet are unevenly spaced in time (Hildebrand, 1977; Alexander and Jayes, 1978a). Most gait patterns can be described in outline by giving two quantities for each foot: the duty factor (the fraction of the cycle time for which that foot is on the ground), and the relative phase (the stage in the cycle at which that foot is placed on the ground) (McGee, 1968). The concept of the duty factor is expanded by Alexander and Jayes (1978a): as it is approximately equal to the step length divided by the stride length, a duty factor of < 0.5, indicates a floating phase (no feet on the ground), whereas a duty factor of > 0.5 indicates a double-contact phase (both feet on the ground).

In the bipedal gait cycle, the stance (or support) phase starts with heel contact and ends with toe off, and the swing phase starts with toe off, through mid-swing, ending with heel contact. In 'normal' human walking, the stance phase usually accounts for approximately 60% of the gait cycle, the swing phase for 40% (Whittle, 1991). There are also two periods of double support (each approximately 10 %), as contact with one foot occurs while the other is still on the ground, except in running, which has a 'floating' phase. (Alexander and Jayes, 1978a). Aside from walking and running, a third type of bipedal gait is hopping. While bipeds such as kangaroos tend to set their feet down in phase with each other when hopping, an interesting 'aberration' is demonstrated by crows, which set their feet down out of phase (Hayes and Alexander, 1983).

In quadrupedal walking, the gait follows the pattern left hind, left fore, right hind, right fore. This provides a good deal of stability, since at very slow speeds, the centre of gravity of the entire body always falls within a triangle of support, only one foot leaving the ground at any given time (Alexander, 1977). With increasing speed, however, there is a change in phase relations between limbs e.g. the LH-RF and RH-LF pattern seen in trotting, and there are periods of static instability where support is lost in the interest of speed (McGee and Frank, 1968). Early work discussed quadrupeds as if they were two bipeds walking behind each other (Alexander and Jayes, 1978a), however later studies showed that this was inappropriate, mainly due to the important contribution made by the muscles of the back and abdomen to swinging the legs (Alexander et al, 1980).

This discussion will now concentrate on bipedal walking gait, although references will be made on points of interest, to other gait patterns. Most of the details are from studies of human gait, although appear to be applicable to birds.

The bipedal gait cycle is illustrated in Figure 1.1

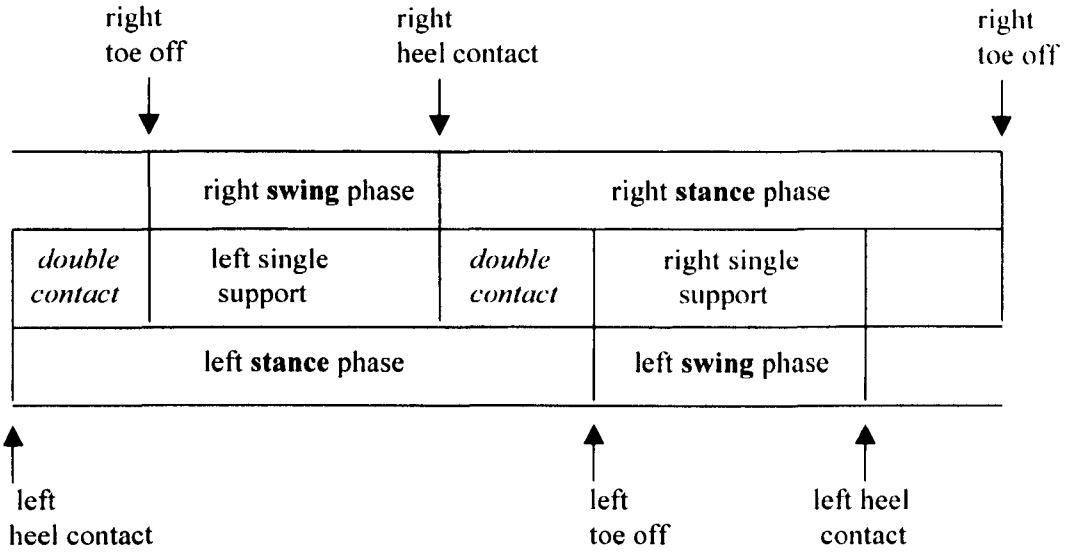


Figure 1.1: Bipedal Gait Cycle

The ground reaction forces arise as a result of the foot in ground contact, however the forces transmitted by that foot are derived from the mass and inertia of the rest of the body (Whittle, 1991). In 1905, Philippson gave a simple description of the four phases of the stance phase, which is still in use today (translation summarised from Wetzel and Stuart, 1977): commencing with foot lift off, all joints flex (F). This is followed by extension beginning in all joints, starting about midway through the swing phase (E1). The third stage starts as the foot touches down, and the knee and ankle (but not the hip) begin to yield or flex under the weight of the body, even while the extensors are still contracting actively (E2). The final stage involves the knee and ankle joining the hip in extension prior to thrust-off (E3). This pattern is maintained despite changes in speed: with increasing speed there is a decrease in stance duration, so that the stages are compressed in time, and the push off force is delivered more quickly (Wetzel and Stuart, 1977).

Energetics of walking

The aim of locomotion is to propel the body (i.e. CG) forwards in as stable and energy-efficient manner as possible. In order to do this, forces must be applied to the ground to raise and accelerate the CG; this is known as the 'external work' of locomotion (work = force x displacement) (Cavagna *et al*, 1977). 'Internal work' is also performed by the muscles to change the kinetic energy of the limbs relative to the CG (Cavagna *et al*, 1977). In order to keep the energy required as low as possible, the CG is moved through a

smoothly undulating pathway of low amplitude (Saunders *et al*, 1953), as abrupt changes in the direction of motion result in high energy costs.

As discussed in the section on biomechanics, a subject is stable as long as a line passing down from the CG remains within the area on the ground supporting it. If it falls outside the area of support, several things can happen, as described by Whittle (1991):

a) in a stable equilibrium, a degree of imbalance produces restoring moments which push the object back into balance i.e. the subject self-rights
b) an unstable equilibrium can ensue, where moments act to increase the imbalance, and the subject falls over
c) a dynamic equilibrium ensues, where there is an unstable equilibrium from instant to instant, but before there is time to fall, the area of support is moved so that the equilibrium is restored.

Normal walking, at moderate speed, represents a situation of dynamic equilibrium – basically, the body falls forward under gravity in the second half of each step, until it is stopped by the next leg entering stance phase (Alexander, 1982).

In discussing the energetics of walking, it is simplest to refer to the path of the CG (the single point at which the whole mass of the body is concentrated; Whittle, 1991), and consider Newton's Laws of motion. Newton's Second Law states that a body will not accelerate unless a net force is acting upon it, and this is the force required to overcome the inertial effects of gravity. Once a body is moving, it will continue to do so at a constant speed and direction, unless acted upon (based on Newton's First Law). Energy is therefore required to do work to propel the body forwards; the energy (Joules) can be either potential energy (Ep), which is 'stored' energy, or kinetic energy (Ek), which is 'movement' energy. This energy originates as ATP (adenosine triphosphate) in muscles (a form of potential energy), and is very inefficiently converted to mechanical energy (< 25%) in response to action potentials (Whittle, 1991). Thus energy has to be put into the system, and so efforts must be made to use it efficiently, and conserve as much as possible.

If we first consider how energy exchange occurs within the system, we can then consider how it can be 'managed' most efficiently. During locomotion, a subject accelerates and decelerates, changing kinetic energy; their centre of mass rises and falls, changing gravitational potential energy, and stretching and recoiling of ligaments and tendons exchanges elastic strain energy (Alexander, 1977; Cavagna *et al* 1977). An 'efficient' gait is

one in which this energy exchange is as complete as possible, a minimal amount of energy being lost and having to be replaced by chemical energy via the muscles - thus keeping the 'net' cost of transport low (Cavagna *et al*, 1977; Alexander and Jayes, 1978b). Adaptation can be seen in the variety of walking styles used, the extremes of which are termed the 'stiff walk' and the 'compliant walk'. (Alexander, 1977; Cavagna *et al* 1977). These basically affect how the CG moves in a vertical direction: irrespective of which type of walk is used, the CG is also displaced laterally twice during each cycle, as it moves over the supporting limb.

In typical slow human walking, the stiff gait described by Alexander (1975b) is used: the leg length remains constant, so the hip moves forward in an arc over the supporting leg, at which point the Ep is greatest, the Ek lowest (as the body is decelerated in the first 'half' of the step) and no elastic storage occurs. Alexander compares the relationship between Ek and Ep in the stiff walk to an inverse pendulum, where the energy exchange is complete, and therefore very efficient. Thus the CG is displaced vertically twice within one gait cycle, with peaks at 25% and 75% of the cycle (mid-stance / swing for opposite legs), and its lowest at 50%, during the period of double support (Saunders at al, 1953).

In the compliant walk, the leg bends when the foot is on the ground so that the hip is at its lowest as it passes over the supporting foot, and so is the Ep. The Ep and Ek fluctuate in phase, therefore no exchange of energy can take place between them, however lots of elastic storage of energy can occur (Alexander, 1975b; Cavagna *et al* 1977). The force is greatest as the hip passes over the supporting foot (in contrast to the stiff walk, where the vertical force is greatest in the double support phase). This type of gait is used by small cursorial mammals (which tend to walk on quite bent legs), as opposed to the straighter-legged non-cursorial mammals (Alexander, 1975 a,b). Although quail walk with a compliant gait, quite obviously bobbing up and down as they walk (Alexander and Jayes, 1978a), many other birds as diverse as ducks and ostriches walk with a more stiff gait (Alexander, 1977; Alexander, 1982). Chickens appear to fall into the latter category, and keep their legs fairly straight during walking. Walking therefore appears to involve basically the same mechanisms in humans and birds, despite the fact that the different organisation of the leg bones gives the appearance that the legs bend in opposite directions (Cavagna *et al*, 1977). Irrespective of how the various forms of energy are exchanged, the important thing is that the <u>total</u> of the three forms of energy fluctuates very little.

Determinants of gait:

As discussed previously, vertical and lateral excursions of the CG are costly in terms of energy, and so various methods are used to minimise the distance the CG moves in these directions. In human walking, for example, the vertical and horizontal displacements of the CG are restricted to about 1 ¾ inches in each direction during the gait cycle (Saunders *et al*, 1953). The techniques (or 'optimisations') used to minimise the excursions of the CG are classically known as the '6 determinants of gait', and were first described by Saunders *et al* (1953). They are simplified by Whittle (1991):

1. Pelvic Rotation - brings the hip joint forwards as the leg flexes, and backwards as it extends (by approximately 4 degrees in either direction). This reduces the amount of flexion / extension of the hip for a given stride length, which means less vertical displacement of the CG (effectively 'flattens' the arc at the start and end).
2. Pelvic Tilt - The height of the trunk is determined by the average height of both hip joints. Thus if the pelvis tilts downwards on the side of the non-weightbearing limb (by an average of 5 degrees) as the hip on the stance phase leg is at its highest, the CG is effectively lowered as it passes over the weightbearing limb. This is only possible if the swing leg shortens enough to clear the ground (by knee flexion and ankle dorsiflexion). Knee flexion effectively shortens the pendulum, also saving energy.
3. Knee flexion in stance phase – as the hip goes from flexion to extension, the hip joint would rise and fall; flexion of the knee shortens the leg as the hip extends, and so 'flattens' the arc in the middle.
4. and 5. Foot and Knee mechanisms: two arcs of rotation occur at the foot. As the heel contacts the ground, the ankle rotates about the foot until the heel begins to lift off the floor. At the start of heel contact, the limb is at maximum length; the knee is fully extended and the foot dorsiflexed. Rapid plantar flexion of the foot, timed at the start of knee flexion, keeps the CG moving forward at approximately the same level for some time, by effectively flattening the arc at the start. The second arc occurs around the forefoot, as the heel is lifted: the second period of knee flexion (associated with heel rise), flattens the arc at the other end.
6. Lateral displacement of the body: the CG is moved over the weightbearing limb by the horizontal shift of the pelvis, or the relative adduction of the hip. If the limbs were parallel, the CG would have to move half the distance between them. Narrowing the walking base through a slight valgus angulation of the knee enables the tibia to be vertical, while the femur inclines towards the median from a slightly adducted hip.

Thus determinants 1 – 5 all reduce the vertical excursions of the CG, and number 6 reduces the lateral displacement; all six acting together produce a much smoother trajectory for the CG, at much reduced energy cost (Whittle, 1991). The relative lengthening of the limb also results in increased velocities being achieved at only slight energy cost, through lengthening the stride rather than increasing the cadence (Saunders *et al*, 1953).

Pathological Gaits:

Abnormal movements can occur if the way a subject can move is limited by some weakness or pathology, or if the subject is using them to compensate for another, underlying problem (Whittle, 1991). While the loss of one determinant can usually be compensated for quite effectively (loss of knee function being the most difficult), it is far less effective where two are lost: the resulting exaggerated motions increase the energetic cost of locomotion, resulting in fatigue (Saunders *et al*, 1953). Several basic types of pathological gait are recognised in humans, again described by Whittle (1991):

a) Lateral trunk bending: towards the side of the stance leg reduces the forces in the abductor muscles and hip joint. Used when the hip is painful (or abductor muscles are weak), and where the walking base is very wide (so that the body is bent to position the CG over the support leg, rather than tipping the whole body over).

b) Anterior trunk bending: at time of heel contact, moves the CG forwards, rather than it passing behind the knee at that point, which would result in knee flexion if the quadriceps are weak.

c) Posterior trunk bending: the reverse of the above, to move the CG backwards e.g. if there is a problem flexing the knee.

d) Functional leg length discrepancies: usually due to neurological problems resulting in the inability to adjust the leg length appropriately at certain stages. Subjects then have to resort to hip-hiking, circumduction etc.

e) External rotation of the leg: alters the direction of the line of force through the knee e.g. to compensate for quadriceps weakness.

f) Abnormal walking base: e.g. deformities such as valgus knee or abducted hip resulting in the feet being further apart (normally 50 – 100mm wide). Commonly, this occurs due to instability or fear of falling, the wider support base allowing for a greater 'margin of error' in positioning the CG.

g) Rhythmic disturbances – in cycle timing, or patterns of limb movement or loading. Most pronounced in the 'antalgic gait' in which the subject spends as short a time as possible on a painful limb, and a longer period on the sound limb. This type of gait

is therefore asymmetrical from side to side, but usually regular from one stride to the next.

In the final part of this discussion on gait, the problems of comparing gait patterns between different sizes of subjects, and different speeds of movement will be briefly mentioned. These are important concepts in the field of locomotion, but will not be discussed in detail as they are irrelevant to the work of this thesis, which considers gait in similarly sized subjects, moving within similar speed ranges.

A major problem arises in trying to compare certain gait parameters between differently sized animals, as various parameters change in relation to body size and speed. Although this is not a problem when studying chickens, it would be if we tried to compare them to ostriches, for example, and is of particular concern in animals that change gait patterns at certain speeds. It has been shown that the speed and stride frequency at which animals move from one gait to another changes in a regular manner with body size (Heglund *et al*, 1974). If the stride frequency is scaled in relation to body mass however, the relationship is extremely similar in bipeds (α M $^{-0.18}$) at top speed, compared with quadrupeds (α M $^{-0.15}$) at maximum trotting speed, and (α M $^{-0.17}$) at maximum gallop (Gatesy and Biewener, 1991).

To address the problem of different speeds, Alexander and Jayes (1978a) devised the 'u' value of 'dimensionless speed' by dividing the actual speed by (gravity x hip height of the animal)^{½} and used this to make comparisons of various aspects of gait. They found that most mammals change from a walk to a trot or run at a value of $\underline{u}$ in the region of 0.8, and from a trot to a gallop somewhere between a $\underline{u}$ of 1.3 and 2. It has been suggested that constant stability in gait requires a duty factor of 0.75 or more, which is only achieved at very slow walks with $\underline{u}$ < about 0.4 (Alexander, 1977). Floating phases can only occur in running and trotting if the duty factor is < 0.5, and these gaits are generally adopted as $\underline{u}$ passes 0.8. Early studies by Muybridge (1957), for example, illustrate an ostrich running at 2.9 m/s (u = 0.9), with a short floating phase.

This concept was taken further by Alexander and Jayes in 1983, when they introduced the 'dynamic similarity hypothesis'. This states that 'different mammals move in a dynamically similar fashion whenever they travel at speeds that give them equal values of a dimensionless parameter, the Froude number'. The aim of adopting this dynamic similarity is to have equal costs of transport, cost being power / (bodyweight x speed). The Froude number is equal to u^2 / gh, where u = speed, g = acceleration of free fall, and h = a

characteristic length. They found that at Froude numbers of around 0.6, men start to run, and crows change from walking to hopping, about the same Froude number at which quadrupeds change from walking to a faster symmetrical gait.

1. 6 GAIT ANALYSIS

Many reviews of gait analysis are available, from the early years (Garrison, 1929, quoted by Whittle, 1991) to more recent times (Vaughan *et al*, 1987; Clayton, 1991; Barrey, 1999). A fascinating history tracing the development of gait analysis is given by Whittle (1991).

The study of motion in animals, known as kinesiology, can be divided into two fields: kinetics (the study of the forces), and kinematics (the study of temporal and geometric characteristics of motion). Most modern gait analysis systems have been developed for one or other field of study, and so it is important to consider which sort of information would be most useful for a particular study, prior to choosing a method. More than one system can of course be used, e.g. a study of locomotion in cattle walking on a variety of different surfaces combined kinetic (Kistler force plate) and kinematic (Selspot LED) systems (Albutt *et al*, 1990).

Gait analysis is a large and constantly evolving field, and the purpose of this review is not to discuss each commercial system in turn, but rather to provide an overview of the different methods of analysis, and compare their usefulness. The various methods are considered in turn, bearing in mind that the 'ideal' gait analysis system should be easy to use, provide reliable, accurate and reproducible data, and most importantly, should not interfere with the subject during data collection (Leach, 1987).

Observational:

This is gait analysis in its simplest, most subjective form. While it is a necessary part of the initial assessment of a subject, the numerous disadvantages of basing clinical decisions on observational analysis alone have been discussed previously. Filming the subject, particularly in several planes, allows closer inspection and repeated review without fatiguing the subject, but the results are still merely descriptive.

Direct Motion Measuring Systems:

These are usually simple e.g. a piece of tape attached to the subject at the heels, which is pulled through an optical reader as the subject walks forward, giving timing and displacement information for both feet (Law and Minns, 1989; Wingfield *et al*, 1993).

Kinematic Systems:

These can be used alone or in combination with complimentary technology such as accelerometers, goniometers or electromyography. The basis of kinematic systems involves marker tracking. Initially, a framework with markers at known positions is filmed, to establish co-ordinates in the field of view. Markers that contrast with the subject's skin and the background are placed at various anatomical landmarks and the subject is filmed moving: the image is captured from various angles by multiple synchronised cameras (usually 6). The images are then either recorded on videotape for analysis, or captured directly on computer using frame grabbers, which convert the analogue video signal into a digital representation of the picture. Each marker point is given a value, and the computer records the co-ordinates. The positions of the markers are then 'tracked' either semi-automatically (where the first frame has to be manually digitised), or the process is fully automated. When the 'known' co-ordinates are fed in, the relative position of the markers on the subject can be calculated, allowing determination of distances, patterns of movement, and joint angles.

Two basic types of system exist, based on the type of marker. Passive marker systems, such as the Peak Performance System (Peak Performance Technologies Inc., Colorado), use markers which reflect light, and so require a source of illumination near the camera, which is often stroboscopic, to avoid smearing. Active marker (or 'optoelectric') systems, such as Selspot (Selspot Systems Ltd., Michigan), use light emitting diodes (LED's), which emit either visible or infrared light. Individual markers are distinguished either by time multiplexing (emissions are turned on and off sequentially), frequency multiplexing (each marker has a characteristic frequency of emission) or shape recognition. A major drawback of active marker systems is the need for power packs to be attached either directly, or by leads, to the subject.

The main problems associated with kinematic systems arise from difficulties in positioning both the cameras and the skin markers. Camera placement must take into consideration both parallax error (which will cause the relative magnification of the limb nearer to the camera compared to the opposite limb), and perspective error (which arises due to changes in the obliquity of the subject's image relative to the camera lens). These can

produce large measurement errors, particularly if the subject's path deviates from being perpendicular to the field of view of the camera.

It is very difficult to place the markers accurately on specific anatomical points, and errors are further compounded by the movement of skin and soft tissues relative to the underlying bones (Winter, 1990). Placing the marker on the skin surface does not equate to the joint centre, as demonstrated by Li *et al* (1993), who used X-rays to accurately locate the designated bony landmarks. Even if placement were accurate, a single marker could not represent the instantaneous centre of rotation (ICR) throughout the full range of movement of a joint where the radius of curvature of the articular surface changes, or a sliding or gliding motion occurs (Clayton, 1991). Marker 'drop out' is also a problem, where a marker is obscured from view, or when marker paths cross or converge. If the marker is seen by at least two cameras simultaneously, its 3D co-ordinates can be determined using Direct Linear Transformation, otherwise estimates can lead to considerable errors, especially when pathological gaits are being studied (Abdel-Aziz and Karara, 1971).

Kinetic Systems - measuring forces and pressures

Force cannot be measured directly, and so has to be estimated: this is normally done by measuring the deformation caused by an unknown force on a material with known force / deformation properties (Cavanagh and Ulbrecht, 1994). Pressure, which is force per unit area (N/m or Pa), can then be derived.

Early attempts to describe load distribution under the foot were made using footprints in clay, however impressions made in deformable substances really describe the shape of the foot rather than the load distribution (Betts and Duckworth, 1978). Simple 'direct-pressure mapping systems' such as the Harris-Beath mat (Footman & Co.Ltd., London) have been used more recently. The mat is composed of thin rubber, with ridges of differing heights that compress under different pressures. When the ridges are inked, and a sheet of paper laid across the top, a subject walking across it causes a differential transfer of ink, producing a semi-quantitative map of the pressure distribution beneath the foot. A more advanced, and cleaner, method involves the use of pressure sensitive film (Fuji Prescale, C. Itoh and Co., New York). These systems are cheap, but 'crude', recording only the peak pressure that occurs at any given point, over a relatively low working pressure range (Betts and Duckworth, 1978).

Many of the modern kinetic systems have been developed from a technique first proposed by Chodera (1957) and later developed by Betts and others (1978,1980a, 1980b) using a system known as a pedobarograph. The recording surface is a glass plate with light totally internally reflected within it. When pressure is applied to a layer of plastic on top of the glass, the light is scattered out of the glass at the points of contact, with an intensity in proportion to the pressure being applied.

Modern force plate systems are of four basic types, the force being calculated from the response of either mechanical springs and pointers, piezoelectric crystals, linear variable differential transformers, or electrical resistance strain gauges (Anderson and Mann, 1994). The forces are usually separated into their '3' components, and the magnitude, direction and duration described for each. While developed primarily for human work, they have been used extensively in equine gait analysis (Goodship *et al*, 1982; Merkens *et al*, 1988; Merkens and Schamhardt, 1994), and increasingly for canine gait analysis (Jevens *et al*, 1996; Budsberg *et al*, 1988, 1996). Although these systems are considerably more sophisticated in the type of information they can provide, they do not show pressure distributions under the foot, unlike the original pedobarograph. Patterns of pressure distribution, along with measurement of regional peak pressures, are still considered by many to be an important area of gait analysis (Bennet and Duplock, 1993; Franks, 1997). A discussion of the various techniques available for the measurement of plantar pressures is provided by Cavanagh and Ulbrecht (1994).

1.7 GAIT ANALYSIS OF POULTRY

As lameness is common in commercial poultry, poultry staff and farmers routinely carry out simple visual gait analysis. The poultry industry has adopted the Bristol Gait Scoring System (Kestin *et al*, 1992) in an attempt to introduce some consistency in describing lameness, however the results of such a purely subjective system will always vary between observers, relying on each person's interpretation of what they see.

In the research field, simple gait studies were carried out on young chicks by dipping their feet in ink and having them walk across absorbent paper (Sheets *et al*, 1987; Farage-Elawar, 1989). From this they could measure stride length and width, and the angle of foot placement between right and left feet. Another approach was used by Abourachid (1991), who made video films of turkeys walking (from lateral and posterior views), with reference spots placed in the field of view. From these films, she was able to measure the duration of various events in the gait cycle. She also measured lateral oscillations of the

body, and found that the centre of gravity was brought over the stance foot to allow equilibrium, resulting in more pronounced lateral oscillations, thereby increasing the energetic cost of walking.

The most complete description of the gait cycle in the chick is given by Jacobson and Hollyday (1982), who studied the behavioural and electromyographic aspects of gait:

The swing phase starts with simultaneous flexion of hip, knee and ankle joints. The duration of this flexion phase differs for each joint - knee flexion is briefest, and knee extension starts during swing, while hip and ankle continue to flex. The ankle then extends (after knee extension has begun), until the foot is placed on ground. Hip extension does not start until the very end of swing / beginning of stance phase.

At the start of stance phase, the knee and ankle begin to flex again, while the hip begins to extend. The ankle flexes slightly, then keeps a constant angle for most of stance. When the body has advanced forward, the ankle begins to extend again (not always the clear transition from E2-E3 described by Philipson). The knee, however, continues to flex for the duration of stance phase, but at a slower rate than during swing phase. The hip extends slightly throughout stance phase.

Throughout the step, the femur points forward, and as the hip joint angle does not change much, the leg does not really move much at the hip joint. Flexing and extending the hip just raises the leg during swing, and minimises the movements of the trunk during stance phase. The caudal movement of the leg during stance is mainly due to movements of the tibiotarsus, caused by knee flexion, so that at the end of the stance phase, the tibiotarsus points backwards. Rapid knee flexion then raises the foot, and although the hip flexes slightly too, the forward pointing femur cannot move the foot forward. The foot is moved forward mainly by the rapid knee extension occurring during the swing phase. Flexion and extension of the ankle during swing raises the foot, and then places it. During stance, changes in the ankle joint only contribute to caudal movement of the foot at the end of stance, and extension in late stance accompanies caudal movement of the tibiotarsus, and assists in propulsion.

The propulsive force is therefore generated by the distal limb in two ways: firstly, by active knee flexion; with the ankle joint stiff, the tibiotarsus and tarsometatarsus move caudally with respect to the body, the leg acting as a lever, the ground as the fulcrum. Secondly, ankle extension during the latter part of stance pushes the body away from the point of contact with the ground - if this contact point is caudal to knee, this force has a forward horizontal component, the leg acting as a propulsive strut.

A detailed kinematic study of joint movement in walking, swimming and airstepping by chicks was also published by Johnston and Bekoff (1992). Having trimmed the primary feathers to visualise the proximal joints, they placed black ink dots on the skin over prominent bony landmarks. The skin overlying the knee joint was sutured to the underlying cartilage to prevent skin displacement in that area causing large errors in marker position. The birds were filmed, and the ink dots 'tracked' on computer, to create stick figures from which segment lengths and joint angles could be calculated. They made the assumption that the hindlimb remains planar (i.e. parallel to the camera lens), partly based on work by Raikow (1985) and Young (1981), who showed that the anatomy of the avian hindlimb is such that the hip, knee and ankle joints move mainly in a saggital plane, abductional, adductional and rotational movements being minimal. As the bird moves forward, the weight is brought over the leg remaining on the ground by rotation at the knee (Young, 1981), the knees of birds being much closer to a transverse line through the centre of mass than the hips (Clark and Alexander, 1975). However Johnston and Bekoff (1992), acknowledged that large errors would still arise if birds themselves deviated from a straight line, and so analysed only those cycles that remained reasonably planar.

An interesting study describing bipedal locomotion in birds and humans was published in 1991 by Gatesy and Biewener, using many of the methods introduced by Alexander and Jayes (1983). They found that smaller bipeds tended to have shorter stride lengths ($\alpha M^{-0.39}$) and higher stride frequencies ($\alpha M^{-0.18}$) than larger bipeds. However, after normalising for size based on the Froude number, smaller bipeds have greater step lengths, limb excursion angles and duty factors, which they suggest is due to their more crouched posture and greater effective limb length. The study showed two particularly interesting contrasts between the gait of birds and humans. Firstly, birds appear to have more compliant limbs, resulting in greater stability and control of head movements, as vertical fluctuations in head height are diminished by increased limb excursion and support time (higher duty factor). Secondly, the location of the centre of mass appears to differ, being well in front of the hip in birds due to the more horizontal orientation of the spine, and the small proportion of the body mass located posterior to the hip. Combined with the more horizontally orientated femur, this results in asymmetrical limb movements being required to keep the supporting foot under the centre of mass: increased angular excursion of the limb occurs mostly by increased retraction, and most movement in the support phase occurs at the knee (flexion ranging from 50-80% compared to 10% at the hip).

While there have been a number of kinematic studies of gait in birds, there appear to have been surprisingly few on the kinetic aspects of gait. Measurement of the forces involved in locomotion would seem to be particularly important when trying to determine changes in gait with lameness. In a study on the mechanics of running by quail, kinematic (marker tracking) and kinetic (force plate) methods were combined to study changes in potential and kinetic energy (Clark and Alexander, 1975). The authors reported maximum vertical forces of 1.4N (bodyweight averaged 1.1N), with 'substantial' longitudinal forces, but small transverse forces. From the kinematic analysis, they calculated the mean excursion of the CM to be 7mm in the vertical direction, and 1.5 mm either side of midline. They also noted that the force records from walking birds were very similar to those of humans, although the longitudinal and transverse forces were larger (relative to bodyweight). Interestingly, a study on a variety of quadrupeds (dogs, macaques and rams) and bipeds (rhea and wild turkey), again using a force plate to calculate mechanical work, found that the force records were 'remarkably similar' between the subjects (Cavagna *et al*, 1977).

In a more recent study, a force-transducing perch was used to measure the reaction forces of small birds at take-off and landing (Bonser and Rayner, 1996). In this study, the forces were found to correlate with body mass, and the landing forces were found to be lower than those at take-off, due to the braking action of the wings.

Considering the various options available for avian gait analysis, there were a number of obvious difficulties associated with using kinematic systems. Marker placement in particular would be problematic, as even if feathers were removed from most of the legs, the hip would still be partially obscured by the wing tips. It would also be difficult to keep the markers in position, as birds tend to preen and peck at 'unusual' things on their bodies. Unlike people, horses or dogs, birds are not easy to train to walk in a predictable way, or at a reasonably constant speed, and the natural lateral sway would complicate calculation of distances from the camera views. Any system requiring the birds to hit specific marks would be particularly useless.

Aside from the potential difficulties of using a kinematic system, it was felt that a kinetic system would provide more relevant information. If a limb is unstable or painful, for example, a subject will be less likely to load it normally: thus the size, rate of development or duration of the ground reaction forces should change, even if the kinematics appear similar. Of the systems available at the start of this work, it appeared that the pedobarograph

was the most suitable to develop for use in birds. The system could be built relatively cheaply, compared to buying any of the other systems; the spatial resolution is high and the data is immediately available for viewing (Betts *et al*, 1980a). It could be designed with a relatively large, sensitive recording surface, which would require minimal control of the subject. It was accepted that it would provide information only on the net force, but this was deemed adequate for this work. As force is a vector quantity, the net force being measured is equal to the sum of all opposing forces acting in the other directions. Plantar pressure could then be calculated by measuring the area over which the force was applied.

Later in the course of this research, access became available to a Kistler Force Plate. This provided the opportunity for a detailed study of other aspects of gait related to those measured on the pedobarograph, such as the individual ground reaction forces and torque. The data obtained using the force plate could be compared with that from the pedobarograph, and used to validate results from the latter system.

1. 8 PEDOBAROGRAPH

In 1934, Elftman used a cine camera positioned under a glass plate to film the pressure distribution during foot to glass contact. This idea was developed by Chordera (1957), who introduced the idea of using internal reflection of light in the glass as a method to measure pressure, and named the system a 'pedobarograph'. In the early 80's Franks, Betts and Duckworth, a group of researchers in Sheffield, computerised the system and developed it as method of clinical gait analysis in hospitals. Most of the following information was obtained from a series of papers published by the group during that period (Betts and Duckworth, 1978; Betts *et al*, 1980a, 1980b, 1980c, 1980d). Although many advances have since been made in force plate technology, the pedobarograph is still valued as a high-resolution method of measuring plantar pressures (Franks, 1997). Most pedobarographic studies of gait have been done on humans, although similar techniques have been used with rats (Walker *et al*, 1994; Clarke, 1995).

Theory:

As discussed previously, force (from which pressure is derived) cannot be measured directly, and is estimated by measuring the deflection or deformation it causes in an object (Cavanagh and Ulbrecht, 1994). The design of the pedobarograph is described by Betts and Duckworth (1978) and illustrated diagrammatically in FIGURE 1.2. The "recording" surface is a glass plate, illuminated at its edges by fluorescent strip lights, and

with a thin sheet of opaque plastic placed on top. The 'deformable' part of the system is the plastic interface, which is pushed against the glass when a subject stands on the plate.

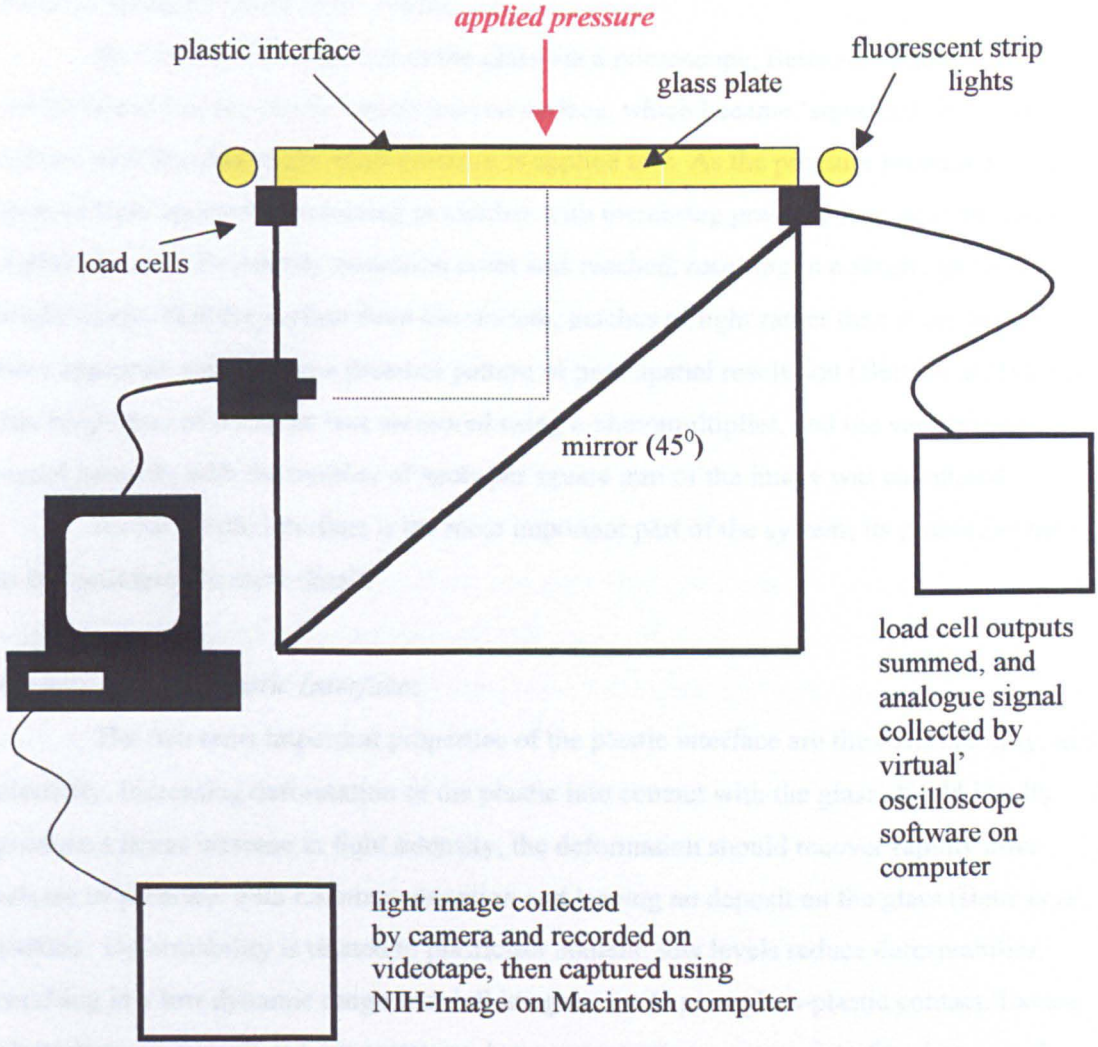


Figure 1.2: Diagrammatic illustration of the pedobarograph recording surface, and associated equipment.

The theory is based on the principle of total internal reflection of light, which occurs within the glass plate when light enters it only through its edges. As glass has a higher refractive index than air, the light rays remain reflected within the top and bottom surfaces of the glass plate. Where an interface is created with something of a higher refractive index than glass, in this case the plastic, the rays are refracted out of the glass at points of contact (Betts and Duckworth, 1978; Betts *et al*, 1980d). The greater the intimacy of contact between the plastic and the glass, the greater the intensity of the scattered light,

which can then be quantified. A camera placed below the glass films the light image, which can then be analysed on computer, to provide information on pressure levels and distributions (Franks *et al*, 1983). The light output of the pedobarograph was found to follow the applied pressure wave very closely, enabling measurements to be made of dynamic footsteps (Betts *et al*, 1980b).

By viewing the underside of the glass via a microscope, Betts and Duckworth (1978) found that the plastic had an uneven surface, which became 'squashed' into intimate contact with the glass plate when pressure is applied to it. As the pressure increased, small spots of light appeared, increasing in number with increasing pressure (and also increasing slightly in size). Eventually saturation point was reached, resulting in a single, uniformly bright image. Had the surface been too smooth, patches of light rather than spots would have appeared, resulting in a pressure pattern of poor spatial resolution (Betts *et al*, 1980d). The brightness of the spots was measured using a photomultiplier, and the variation in signal intensity with the number of spots per square mm of the image was calculated.

As the plastic interface is the most important part of the system, its properties have to be considered in more detail.

***Properties of the Plastic Interface*:**

The two most important properties of the plastic interface are the deformability, and elasticity. Increasing deformation of the plastic into contact with the glass should ideally produce a linear increase in light intensity, the deformation should recover rapidly after release of pressure, with no image retention and leaving no deposit on the glass (Betts *et al*, 1980d). Deformability is related to plasticiser content: low levels reduce deformability, resulting in a low dynamic range and dull images, due to poor glass-plastic contact. Excess plasticiser can extrude at high pressures, however, creating a greasy interface between the glass and plastic, resulting in saturated images at low pressures, again over a low dynamic range (Betts *et al*, 1980d).

Elasticity is defined as 'a property of materials in response to stress, indicating the degree to which strain disappears from a material when the stress has been removed' (Morris, 1992). In a linearly elastic material, the strain energy is fully stored during loading and recovered during unloading. With linearly visco-elastic materials such as plastics, however, some energy is 'lost' within the material as it is stressed, so the stress and strain (loading and unloading) curves are out of phase, i.e. show 'hysteresis' (Biewener, 1992). With viscoelastic materials, the phenomena of 'creep' and 'continuous deformation' must be considered when prolonged loads are applied. When strain increases with time, despite

the fact that the stress is kept constant (a phenomenon known as 'creep'), continuous deformation of the plastic into contact with the glass will result in an increasing light output for the same applied pressure. (Betts *et al*, 1980d; Franks and Betts, 1988). This could cause problems in producing calibration curves of dynamic pressures, particularly if high or prolonged pressures are likely to occur. Calibration should therefore be standardised by applying pressures from zero, and allowed them to return to zero before repeating (Betts *et al*, 1980d).

Tests on a range of plastics found that while they all show a degree of creep and hysteresis, it is not significant when the strain is applied for less than half a minute, well within the time of a normal footstep (Betts *et al*, 1980d; Franks and Betts, 1988). Ilfospeed pearl finish photographic paper was found to be the most suitable, showing little viscoelasticity, tackiness or other hysteresis-like effects, and producing a linear response in light output to applied pressure.

Calibration of the plate:

Having considered the physical properties of plastic / glass interface, the relationship between the applied pressure and light output has to be defined for each system. Calibration of the system is relatively simple for static measurements, but considerably more difficult for dynamic studies.

For static calibration, Betts and Duckworth, (1978) initially used a pressurised air cell connected to a compressed air cylinder, which was clamped to the top surface of the glass plate. Various pressures were applied, and measured using a pressure transducer and amplifier. The 'light' image was recorded, the image intensity measured using a photomultiplier, and a calibration curve plotted of light output against the applied pressure. Most tests produced a linear relationship, except at the extremes of the calibration curve. Due to the uneven surface texture of plastic, 'high points' tend to dominate contact at very low pressures, while at the other end of the scale, saturation eventually occurs when maximum intimate contact with the glass has been reached (Betts *et al*, 1980d). Although some useful information can be gained from static measurements, many gait abnormalities are due to abnormal function and will only become apparent from dynamic measurements (Betts *et al*, 1980b).

As discussed previously, the plastic interface behaves differently depending upon whether pressure is applied statically or dynamically. It is therefore inappropriate to apply static pressures to dynamic situations. Several methods of dynamic calibration were tested. Betts *et al* (1980b) used a force transducer attached to a flexible bar with a soft rubber

sphere attached to its underside. The sphere deformed evenly to produce a uniform load distribution, resulting in an even light output from the plate. Various load impulses were applied, with force / time characteristics corresponding to those encountered under the feet. The design of the system was improved in 1983 by mounting the pressure plate on force transducers, enabling dynamic frame by frame calibration, using the integrated light output values and the sum of the force transducer outputs (Franks *et al*, 1983). Although various pneumatic techniques were also tested, it was found to be difficult to reproduce the necessary frequency and pressure requirements to mimic those of walking (Franks and Betts, 1988). The simplest method, which was described by the aforementioned authors, was to use a 'thumb press' or 'heel stamp' to obtain a series of images.

Dynamic calibration showed that although the response to increasing pressures was rapid, there was a small lag in response to decreasing pressures - in the order of 10 - 15 milliseconds to go from maximum light output to near zero. Thus the decrease in image brightness will lag slightly behind the decrease in pressure, so that lower pressures may result in brighter than 'expected' images. This can complicate calibration of adjacent areas which may be subject to increasing and decreasing pressures simultaneously (Franks *et al*, 1983; Franks and Betts, 1988). This error in assuming linear calibration for all points in a particular image has been mentioned previously. However, Franks *et al* (1986) suggest that as the difference between normal and abnormal pressures is usually significantly large, clinical use does not require very high accuracy. This point is debatable: while subtle differences may be less important in preliminary studies, it would limit the usefulness of the system in more sophisticated decision making e.g. for pre-operative decision making, or post-operative monitoring.

In general, the pedobarograph proved to be a very useful system in the field of human gait analysis, particularly in the study of gait problems in diabetic patients. It was found that although the distribution of pressure under the foot varies between individuals, peak pressure levels normally remain within reasonably narrow limits (Betts *et al*, 1980c). The ratios of peak pressures and loads from heel to forefoot and from lateral to medial borders of the foot were found to be approximately unity, and it is suggested that these ratios are a useful method of assessing the normality of the pressure distribution (Betts *et al*, 1980a). Although useful information can be gained from examining the pressure distribution under the standing foot, many abnormalities are functional rather than anatomical, and so the system must be calibrated for dynamic studies, as well as standing pressures (Betts *et al*, 1980b). Pressures during standing in humans have been quoted as

ranging from 0-5 kg / cm ^{2} , while those of walking can increase to around 20 kg / cm^2 (Franks and Betts, 1988).

As pressure is derived from force, both speed of movement and bodyweight of the subject should affect the pressures developed under the foot (Betts *et al*, 1980b; Duckworth *et al*, 1982). Some researchers have standardised the scale of the footstep e.g. to 0.8 second, in order to make comparisons between different speeds, and found that the range of normality for pressure variations over a particular area is reasonably constant over the range of normal individuals (Betts *et al*, 1980b; Duckworth *et al*, 1982).

Bodyweight might also be expected to influence pressure under the foot, so that it might be more appropriate to express peak pressures in terms of % body weight. While this is routinely done for force, Duckworth *et al* (1982) suggest that it is the absolute pressure acting on an area which is important in terms of causing damage. They found that there is only a slight increase in peak pressure under the foot from childhood to adulthood, suggesting that the foot functions in such a way that the loads are distributed to keep pressure within a relatively narrow limit. This could also be brought about by changes in the soft tissues of the foot (Cavanagh and Ulbrecht, 1994). Work by Pontious *et al* (1993) suggests that the hyperkeratosis often present under the first and fifth metatarsals may be a natural effort to protect these plantar regions from excessive pressures (although generally hyperkeratoses increases plantar pressures, a diffuse hyperkeratosis will minimise excessive pressures by distributing the forces over a larger area). Considering the thickened epidermis of the bird's foot, it will be interesting to examine how this affects peak pressures, and pressure distribution.

Several papers have been published on the reliability and repeatability of measurements made on pedobarographic systems. The greatest variability was found in measurements taken on either border of the forefoot, and smallest for measurements from the heel (Holmes *et al*, 1991). This variability was considered from a different perspective by Bennet and Duplock (1993), who suggested that those structures with a large degree of functional variability have the greatest functional role in pressure distribution. They proposed plantar pressures should be analysed from two perspectives: to establish the structural order of pressure, and to compare the level of maximum pressure relative to the 95 percentile limits.

1. 9 KISTLER FORCE PLATE

The technical details of the Force Plate are described in the General Materials and Methods section (Chapter 2), with a brief outline and literature review presented here.

In 1880, the Currie brothers discovered that when force was applied to certain crystals (such as quartz), producing minute changes in shape, they responded by generating an electric charge (the 'piezoelectric' effect). It was also found that depending on the orientation of the 'piezoelectric' crystals, they responded to different forces. Such crystals form the basis of the Kistler force plate, where quartz discs, cut in certain directions, respond to either pressure (longitudinal effect), or shear (shear effect), to produce a signal in proportion to the applied force (Kistler Commercial Data).

The Kistler force plate measures the magnitude, direction and duration of the ground reaction forces (GRF's) produced during movement, separating them into their various components. While most of the work has been done in the human field, equine gait analysis is also very advanced, however GRF analysis in small animals is still relatively novel (a good review is provided by DeCamp, 1997). Most of the studies on animals have concentrated on the GRF's, with little published on the other variables. Ground reaction force data is normally presented as a force-time graph, however analysis of waveforms is difficult and usually involves Fourier analysis (Alexander and Jayes, 1980). It is more common to use discrete data points to determine the individual forces, with reference to peak forces, loading rate (slope of line on force/time curve) and total load over time (integral of force-time curve) (Budsberg *et al*, 1988; Rumph *et al*, 1993; DeCamp, 1997).

It is important to note that the terminology used in kinetics is not standard between studies, even when the same forces are being described. The conventions proposed by Kistler are used in this study.

A 'typical' ground reaction force trace of a footstep from a walking human is illustrated in Figure 1.3.

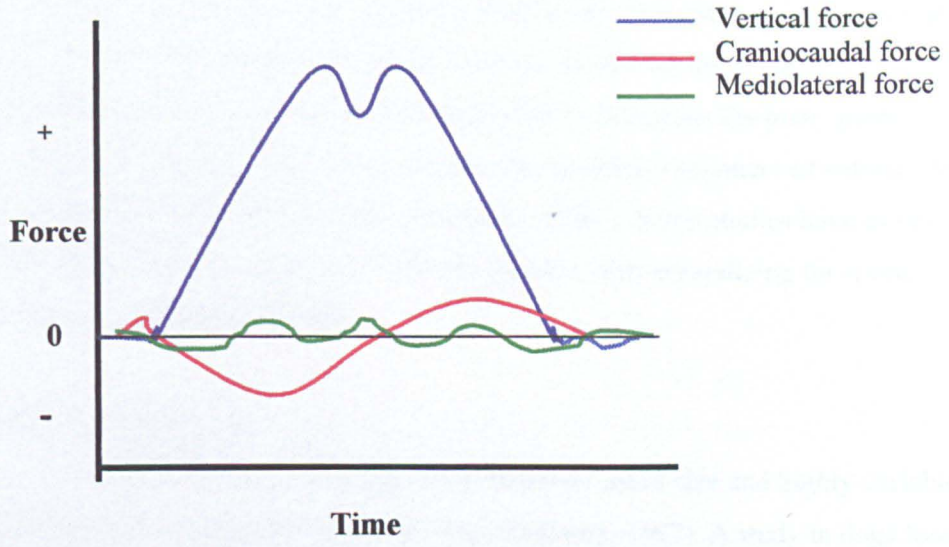


Figure 1.3: 'Typical' ground reaction force trace from a footstep of a walking human.

Vertical Force (Fz).

This is normally the largest of the GRF's: it is related to bodyweight, and is usually positive, negative only in theory if walking on an adhesive surface. This force rises rapidly as the foot contacts the ground and the body mass is accelerated upwards: an initial spike may be seen on the graph, the heelstrike, just preceding the general rise (Lord *et al*, 1986; Winter, 1990). The trace reaches a peak as the bodyweight is transferred onto the limb, then dips slightly, due to a slight bending of the knee unloading the plate in mid-stance, before reaching a second peak, again greater than bodyweight, during push off (Winter, 1990). The force then drops to zero as the foot is unloaded. The two maxima are obvious in walking, but not in running. The first peak is thought to occur when the metatarsal heads have made contact with the ground, and the second when the toes are in contact, and the heel is leaving the ground (Lord *et al*, 1986).

The mean vertical component of force over a complete cycle of leg movements must equal the bodyweight (Alexander and Jayes, 1978a; Alexander, 1980). In the human, maximum peak forces of around 120-150% bodyweight are seen at the walk (Whittle, 1991). Forces are obviously greatly increased in running and jumping, but these types of gait will not be considered here.

Horizontal or craniocaudal force (Fy)

This is usually divided into braking and propulsive phases (Budsberg *et al*, 1987). Initial braking occurs as the foot contacts the ground and deceleration occurs, represented by a negative trace due to backward horizontal friction force between the foot and the floor (Winter, 1990). This peaks, then declines to zero. It then becomes positive near midstance as the plantar flexors act, causing the foot to push back against the plate, propelling the body forward (Winter, 1990). Consequently, the forward component of velocity fluctuates in the course of the step (Alexander and Jayes, 1978a). Some studies have examined the craniocaudal forces in terms of %'s of stance phase, thus normalising for speed (McLaughlin and Roush, 1994).

Mediolateral force (Fx)

This is rarely described, due to the relatively small size and highly variable nature (Goodship *et al*, 1982; Budsberg *et al*, 1987; DeCamp, 1997). A study in dogs found that the usual mediolateral force pattern was directed laterally in the forelimbs, but variable in the hind (Rumph *et al*, 1994). For steady walking on level ground, however, Fx must obviously have a mean value of zero (Alexander, 1980).

Many factors influence the results of forceplate studies, not all of which can be controlled. Factors such as body conformation, limb length and angulation are difficult to control, whereas gait, speed, direction, familiarity with surroundings and procedure, and number of tests should be standardised as far as possible (Winter, 1985; Rumph *et al*, 1993). The contribution of variance from different subjects (dogs), handlers and trials on various aspects of the ground reaction forces was studied by Jevens *et al* (1993). They found that while the variance due to the handler is minimal (1-7%), the variance attributable to dogs (14-69%) and trial repetition (29-85%) can be considerable. In contrast, Kadaba *et al* (1989), found that with subjects walking at their natural or preferred speed, gait variables were quite repeatable, particularly within a test day compared to between different test days (although they acknowledged that patients with gait disabilities might show lower levels of repeatability).

The two most important factors affecting the GRF's are bodyweight, and speed. A study by Budsberg *et al* (1987) showed that GRF's correlated with all morphometric measurements, but found bodyweight to be the most accurate and reproducible value. They found significant negative correlations between peak vertical forces and bodyweight,

humeral, femoral and paw length, during walking in normal dogs. Significant positive correlations were found between the vertical (support) impulses and the morphometric measurements however, stance phase correlating directly with physical size. This apparent anomaly is explained as a mechanism to reduce the peak loads on the musculoskeletal system by increasing the time over which the impulsive forces are exerted, in agreement with other studies (Riggs *et al*, 1993; McLaughlin and Roush, 1994; DeCamp, 1997). In most studies, force is normalised to bodyweight, to remove the effects of mass (Winter, 1985; Budsberg *et al*, 1987; Kadaba *et al*, 1989; McLaughlin and Roush, 1994).

It is less simple to control for speed. Peak vertical forces were found to increase, and vertical impulses to decrease, with increasing speed (McLaughlin and Roush, 1994; DeCamp, 1997). Significant changes in peak vertical force occurred with increases of speed of < 0.6m/s (Riggs *et al*, 1993). Speed of locomotion is particularly important in animals that show pronounced changes in gait with increased speed, e.g. dogs or horses changing from walk to trot. In their 1994 paper on Greyhounds, for example, McLaughlin and Roush found that while there was no significant difference in % contact time spent braking or propelling at two walk velocities, braking % increased and propulsion % decreased significantly in the hindlimbs between slow and fast trots.

A significant negative correlation was found between stance time and velocity by McLaughlin and Roush (1994, 1995). They also found that while vertical, braking and propulsive peak forces all correlated negatively with stance time, vertical, braking and propulsive impulses all correlated positively i.e. at slower speeds, the stance times are longer, with greater impulses but lower peak forces. These findings agree with the proposition by made Budsberg *et al* (1987), that the increase in stance time will distribute the force over a greater period and so minimise the peak loads on the musculoskeletal system.

Despite the significant correlation between velocity and stance time, some studies have suggested that peak vertical force and impulse correlate more closely to stance time than velocity, prompting some workers to suggest that GRF's should be examined in the context of stance time ranges, as well as speed ranges (McLaughlin and Roush, 1994, 1995). Other studies have 'normalised' certain events in the gait cycle to 100 % of stance time (Winter, 1985; Budsberg *et al*, 1988; Kadaba *et al*, 1989). In contrast, other workers feel that this technique should only be applied to studies on 'normal' animals, as lame animals usually show decreased stance times (DeCamp, 1997). While Merkens *et al* (1988) found no relationship between speed and degree of lameness in horses allowed to walk at

their own natural speed, they did agree that disproportionate changes in stance time can occur in the lame and sound limb, making comparisons difficult. Most authors therefore limit speed to certain ranges, and draw conclusions accordingly (Riggs *et al*, 1993; McLaughlin and Roush, 1994, 1995).

The relationships between the ground reaction forces and lameness are complex. A study on rheumatoid arthritis found gait velocity and peak forces to be significantly reduced, with a disruption of the normal sequence of heelstrike/foot flat / toe off (Lord *et al*, 1986). Ground reaction forces were measured in dogs before and after unilateral hip replacement, and it was found that loading rates of the treated limb were significantly increased, although they never reached those of the untreated limbs (Budsberg *et al*, 1996). This illustrates the importance of having pre- and post-operative comparisons, as well as treated vs untreated ones. A study on the GRF's before and after anterior cruciate ligament (ACL) repair in dogs showed that all the force-plate measurements, except for limb loading time, were significantly less in the affected limb prior to surgery (Budsberg *et al*, 1988). Another study compared the outcome of two different methods of ACL repair using GRF measurements, and found that peak vertical forces and impulses were significantly decreased at all times in the control and 'under-and-over' technique; however in dogs that had modified retinacular imbrications, the vertical peak force and impulse had returned to pre-operative levels by 20 weeks (Jevens *et al*, 1996).

Two interesting questions were raised by these studies: how well does the clinical assessment of the subject correlate with the results of the force plate analysis, and what is the most appropriate control to use in a lame animal?

Comparisons of subjective lameness scores with GRF data revealed significant correlations (Spearman's Rank correlation coefficients), however subsequent calculation of the coefficient of determination (i.e., the ability to predict y given x by squaring the r value of the correlation) indicated that subjective evaluation is limited, suggesting that unblinded bias of clinicians can be substantial (Budsberg *et al*, 1996). However, significant relations were found between peak vertical forces (and impulses) and clinical grade of lameness by Jevens *et al* (1996), who suggested that grading certain types of lameness e.g. stifle lameness, may be easier than others, e.g. hip problems.

The second point related to the suitability of using another limb in the same animal as a 'control'. In his review of gait analysis in dogs, DeCamp (1997) states that the contra-lateral limb should not be used as a normal control in studies where any significant

lameness is present, without acknowledging that the forces will not be normal and that they can change with the degree of lameness of the affected limb. This is demonstrated in studies by Budsberg *et al* (1988), and Jevens *et al* (1996), who found that after surgery for ACL repair, there was a small but consistent decrease in the corresponding clinically normal hindlimb measurements, suggesting a redistribution of force and weight back to the affected hindlimb. A study looking at the vertical GRF distribution during experimentally-induced synovitis in dogs also found that when arthritis resulted in diminished function in a hindlimb, there was a sufficiently large compensatory response in the other limbs to suggest a dependant relationship between severity of lameness in one limb and force compensation in the other (Rumph *et al,* 1993). Although these authors agreed that the pattern of force redistribution would probably vary based on the severity and duration of lameness, and presence of lesions in other limbs, they concluded that the opposite or other untreated limb should not be used as a control in acute lameness studies.

In contrast, a study of the GRF's pre- and post-unilateral hip replacement, found that while there was significantly increased loading rate and loading function of the treated limb, the untreated limb did not have changes in peak vertical force or impulse over time (Budsberg *et al*, 1996). The authors suggested that in a more chronic injury, "the neuromuscular system adapts or is reprogrammed via sensory information from the unstable or painful joint that protects the affected limb by decreasing loading ... when both hips are affected and pain persists for an extended period, the CNS adapts and diminishes load bearing on both hindlimbs." It is also possible that with more acute lameness in dogs, some redistribution of force may occur to the forelimbs (Jevens *et al*, 1996).

Although all these studies have been on quadrupeds, the results are relevant to bipeds, where the effects may be even more pronounced, the forces being able to be redistributed between only two legs.

1. 10 AIMS OF THIS STUDY

The work contained in this thesis was designed to achieve the following:

1. To develop and test an objective system of gait analysis for poultry.
2. To objectively describe gait in Brown Leghorns and broilers, quantifying various gait parameters.
3. To investigate scaling effects in modern broilers, with respect to locomotion
4. To investigate the possible role of pain in broiler gait.

CHAPTER 2:

GENERAL MATERIALS AND METHODS

2 : 1 PEDOBAROGRAPH

The pedobarograph was built from 'first principles', and so much of the initial research involved the development and testing of the system, which is described in detail. The next section describes the technical aspects of the Kistler force plate, along with the methods used to process the large amount of data that was produced. The gait parameters under study are then explained, followed by a description of the birds used in the work and an explanation of the statistical methods applied to the data analysis.

2 : 1 : 1 DESIGNING THE SYSTEM

The system can be considered as being in two parts. One part is designed to produce 'light images' of the foot on the plate and capture them on computer for image analysis. The other part of the system is designed to indirectly measure the applied forces. The results of each 'part' are then combined to calculate pressures, and describe their distribution under the foot.

The pedobarograph set-up is illustrated in Figure 2.1

The recording plate was built into a runway (length 200 cm, width 40 cm, height of sides 42 cm). The plate was set in the middle of the runway, flush with the surface, allowing the bird to reach a steady speed and maintain it across the plate, before having to slow down.

Image Analysis

The recording surface consisted of a white float glass plate (540 mm x 400 mm x 6 mm), with fluorescent strip lights (Thorn 18 inch, 18 watt) positioned along the two longest edges (see Figure 1.2). White float glass is of ophthalmic quality, having fewer imperfections than regular 'window' glass (Spanoptic, personal communication, 1995). The glass plate must be free from surface deposits, as any smearing, for example, creates an interface, preventing accurate reproduction of the pressure patterns. The fluorescent lights were enclosed in plastic tubing so that all the light was directed into the edges of the glass plate to produce total internal reflection of the light within the glass.

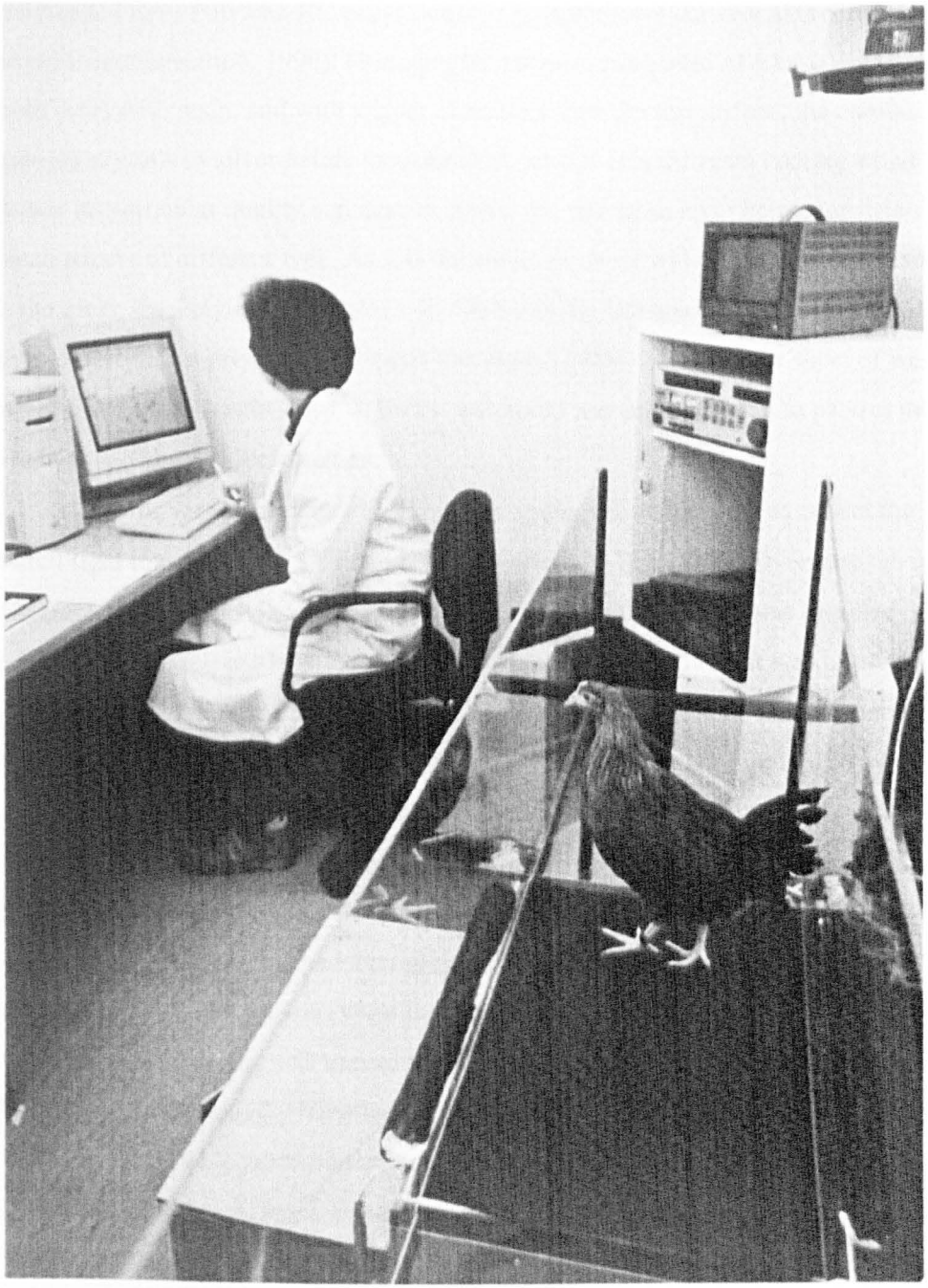


Figure 2.1: Pedobarograph testing set-up.

A sheet of unexposed, processed photographic paper (Kodak Polymax RC paper, surface E, lustre, 45 x 35 cm) was placed on the upper surface of the glass, with the emulsion side downward. The Ilfospeed paper used in the original system built by Franks, Betts and Duckworth (FBD system) is unfortunately no longer made; however Kodak recommended their Polymax RC paper (surface E, lustre) as a suitable alternative (Kodak, Personal Communication, 1996). Photographic paper is composed of a base paper, coated on both sides with resin, and with a layer of emulsion on the top surface, the emulsion containing crystals of silver halide suspended in gelatin. It is the resin coating which gives the paper its particular quality e.g. matt or lustre, the emulsion layer being consistent between papers of different type. As it is the emulsion layer which deforms into contact with the glass, the Polymax RC paper was similar to the Ilfospeed paper in this respect, and so should perform as predicted by Franks and Betts (1988). A thin cover sheet of washable, lightproof material (BenchGuard, J. Bibby and Sons) was placed on top to protect the film and reduce background illumination.

A mirror was positioned at a 45-degree angle below the plate, to reflect the scattered light image to a CCTV camera (Panasonic WV-BP3101B). To ensure the image was accurately reproduced, the automatic gain control on the camera was disabled, and it was fitted with a fixed iris lens. Because the lens aperture was fixed, it was important to check that light did not saturate the camera. Franks (1997) comments that pressures of up to 15 kg / cm^2 should not saturate the camera, producing a maximum output of 0 - 1 or 1.4 volts. Most cameras, however, only read up to 0.7 or 0.8 volts, which would result in saturation at low pressures if the aperture was set too wide. This was checked by linking the analogue output of the camera to an oscilloscope, and measuring the voltage change. The Panasonic model used in this pedobarograph gave an output up to 1V, and did not saturate over the range of thumb pressures applied.

The camera signal was transmitted to a Sony monitor (PVM-9040ME) for real-time viewing, and simultaneously recorded on a Panasonic S-VHS recorder (AG-7355) which had its automatic gain control disconnected to prevent any adjustment of the image quality. The video recorder has a steady freeze-frame facility, which enables advance by single frames during later review and analysis. The images were transferred from video to a PowerMac 8100 /110 computer for analysis, using a Scion LG-3 frame grabber card. The card was installed in the computer and contains a 'flash-converter', which converts the incoming analogue signal from the video into an 8-bit digital value (Scion commercial literature). Eight-bit scaling allows 256 grey levels to be produced, and the normal convention on the Mac is for white to be given a value of zero, while black is given 255.

These two 'extreme' values are reserved by the computer for text however, leaving values from 1 to 254 available for image analysis functions. The 'invert pixel values' function in Image was used to reverse the standard convention and have black given a value of 0 and white given 255, as this made the subsequent calculations of light intensity easier (increasing brightness resulting in increasing pixel value). The resulting digital representation of the picture had a resolution 640 x 480 pixels. Scion Image (version 1.57), a version of the public domain image acquisition and analysis software NIH Image, was used with the card.

The rate at which the frame grabber captures frames was set manually, based on the speed of movement of the subject. If too low a frame capture rate was used, information was lost; a fast frame rate captured great detail, but only of a small part of the overall 'run'. The frame grabber used in this work had 16 MB in the expandable frame buffer, enabling it to capture and store up to 32 frames 'real time' (digitising speed of 1/30 second).

To enable measurements to be made on the images, the system was spatially calibrated by placing a grid of known dimensions on the surface of the plate. The image of the grid was captured by the computer, and the known distances measured using tools available in the NIH software. Using the 'calibrate' function, a measurement was made and the distance automatically recorded in pixels. If the known distance e.g. in millimetres (mm) was then entered, the number of pixels per mm was calculated, and subsequent measurements using the measurement tool are automatically given in millimetres. Measurement error was calculated by taking duplicate measurements (n=100), and calculating the variance between the logarithms for each length. The coefficient of variation of measurement error was found to be less than 1%, and distortion by the camera (due to perspective and parallax errors) was found to be minimal across the recording area.

Force Measurement

The system was designed with a 2 kg load cell (RS 632-736) under each corner of the glass plate to measure the applied force. The output from each of the load cells was summed and connected to a strain gauge amplifier (RS 846-171), to create an analogue signal, voltage being proportional to the load applied to the plate. The output of each cell was independent of the point through which the load acts, with a total error of 0.025% of the total load (RS commercial literature).

Static calibration was quite simple, and involved placing objects of known mass on

various parts of the plate and recording the voltage output produced in response to the downward force being exerted by the mass. The analogue signal was initially connected to a 3476A Digital Multimeter (Hewlett Packard), and the results showed a linear relationship when force was plotted against voltage. When the loads were applied dynamically however, there was a delay in the changes being reflected on the LED display. This made it difficult to synchronise the light image with the correct voltage reading, making calibration inaccurate, and so the system was modified to cope with dynamically applied loads.

The problem of producing a 'real-time' record of the voltage output was solved by using a software package, Labview Virtual Bench (Version 2.1.1, National Instruments). This was installed on a second computer (Viglen genie PCI), along with a DAQ board (ATMIO16E10, National Instruments) to collect and digitise the analogue output from the load cells. The software has a 'virtual oscilloscope' package, which produces a dynamic 'real-time' trace of the voltage output from the load cells on the computer screen. To enable synchronisation of the light image with the voltage output, a small light bulb was positioned in the field of view of the camera. The bulb was connected to a switch that also produced a deflection on a second trace on the virtual oscilloscope (a square wave), at exactly the same time as the light was switched on (see Figure 2.2). The spot of light on the videotape could then be synchronised with the voltage trace from the load cells.

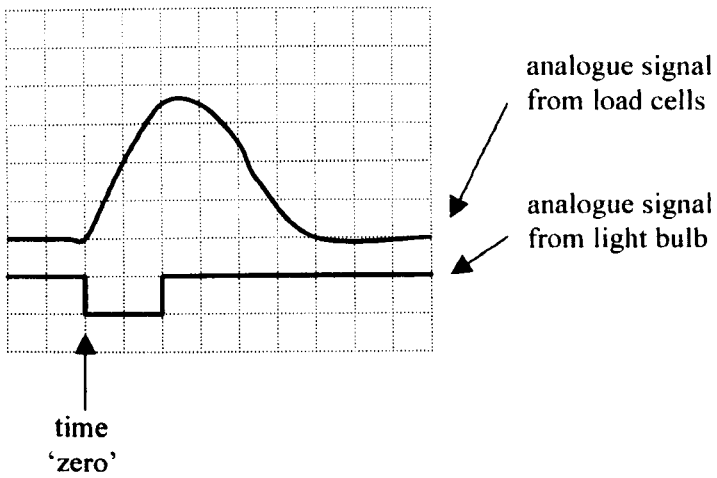


Figure 2.2: Synchronised traces on 'virtual' oscilloscope screen.

The system was tested to calibrate voltage output in response to load, by placing objects of known mass (increasing at 10g intervals) on the plate. The response was linear, and described by the formula: **mass = ('voltage'+4.979) / 0.547.** It was established that the readings were not significantly different wherever the mass was placed on the plate, and were consistent whether the strip lights and camera were switched on or off.

The next step was to determine the relationship between the 'light image' data, and the force data, to calibrate the system.

2 : 1 : 2 CALIBRATING THE SYSTEM

Initially, a calibrator similar to the pressurised air cell described by Betts *et al* (1980a) was borrowed from Strathclyde University biomechanics department. This was used to calibrate their pedobarograph for human gait studies, and had a large surface area, which was pressed into contact with the glass to produce the pressures. Pilot trials with birds indicated that pressure is not evenly distributed under the bird's feet: areas of high pressure are concentrated under the small digital pads, producing very bright images on the pedobarograph (see Figure 2.3 a and b).

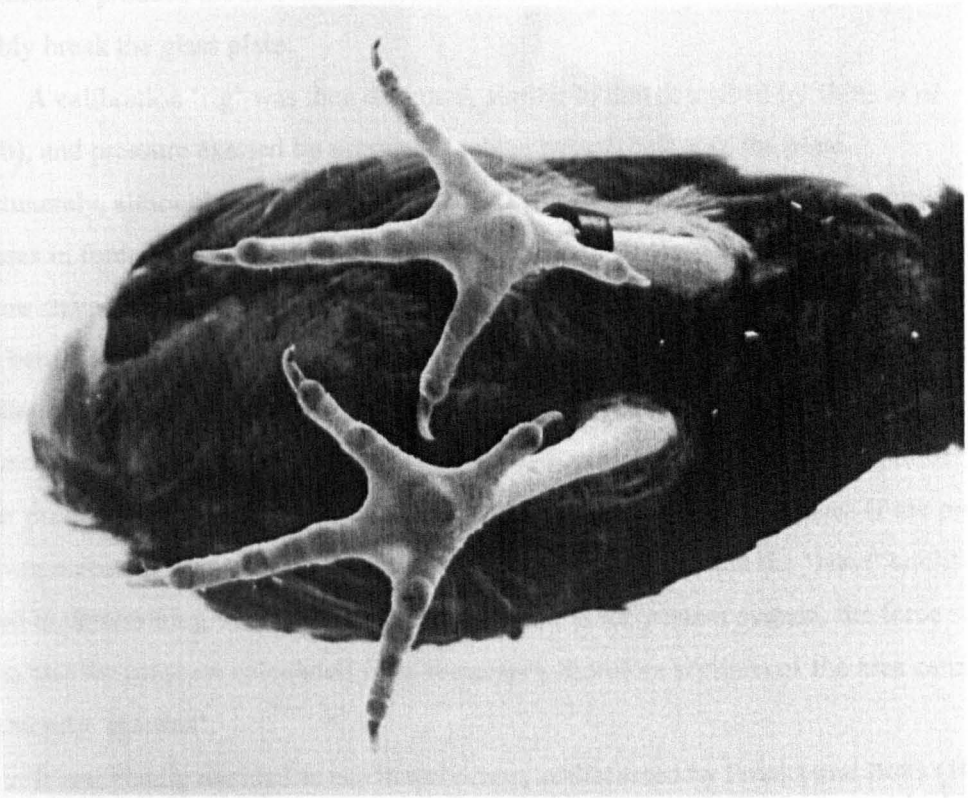


Figure 2.3 (a): Underview of a chicken's feet.

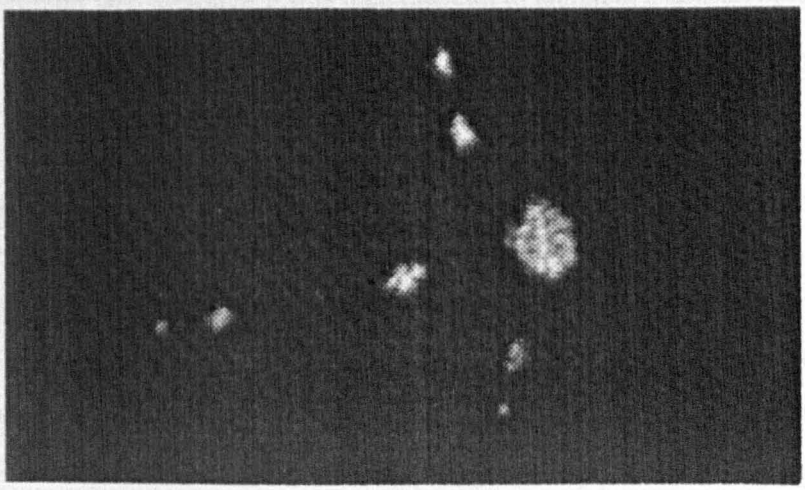


Figure 2.3 (b): Image of a chicken's foot on the pedobarograph, showing the bright points.

Unfortunately the large surface area of the calibrator meant that it would require a large force to produce the relevant pressures, which would overload the load cells, and probably break the glass plate.

A calibration 'rig' was then designed, similar to that described by Betts *et al* (1980b), and pressure exerted by pressing a rubber squash ball onto the plate. Unfortunately, although this initially resulted in producing increased pressures, further increases in force resulted in the ball starting to collapse, so that its area increased and the pressure stayed about the same. Filling the ball with water, or silicone rubber, maintained its shape better, however it still collapsed at relatively low pressures. A variety of other objects, including pencil rubbers, snooker cue tips and rubber "eyeballs" proved equally unsuitable. With most of these objects, the problem of edge effects also occurred, where a rim of greater pressure creates a bright band around the edge as the object collapses. If the pressure was even across the rest of the area, and that pressure is known, then the 'band' could be ignored in determining the mean pressure. However, in the present system, the force was known, and the pressure calculated from force/area, therefore sections of the area cannot be conveniently 'ignored'.

It was finally decided to use thumbprints, as discussed by Franks and Betts (1988). In this way, it was possible to exert variable pressures of a similar duration to those produced during a step, the tissue behaves not unlike a bird's foot, and no edge effects occur. Initially, increasing force causes an increase in surface area (as the thumb 'squashes'), thus the pressure stays the same. Pressure will then increase as surface area has reached maximum, and although it was then difficult to maintain a constant pressure, this was not necessary as the video was synchronised with the force readings being captured on oscilloscope.

The system was switched on for 30 minutes prior to starting data collection, to allow it to 'warm up' and equilibrate. As well as allowing the lights and load cells to stabilise, temperature stability of the plastic is important. Although the stability of the plastic was found to be reasonably consistent over a range of room temperatures, high temperatures could introduce errors of 10 - 15%, if the plastic becomes softer and so are more easily deformable (Betts *et al*, 1980d).

Background illumination:

The recording surface area is determined by the size of the plastic interface (45 cm x 35 cm). While spatial measurements were made over the whole of this area, the footstep nearest the centre of the recording area was chosen for pressure measurements. Although most of the light was internally reflected within the glass, the recording surface was not completely black, and there was some background light. The background light was dealt with in two ways. Before each run, an image of the plate was captured, and a series of 20 measurements made to calculate the average background level within the 'working area'. Two processes were carried out prior to analysing an image. Firstly, the image was magnified to enable the edges of the footprint to be distinguished as individual pixels. The 'density slice' function in the software was then applied. This highlights pixels within a specified range, the high end of the range is left at 254, and the low end set to ensure that it includes all the 'low intensity' pixels at the edges of the foot image (usually around a value of 65). As the pixel value of the rest of the plate falls well below 65, this effectively 'isolates' the footprint for analysis. Measurements were then made from the footprint, and the predetermined background level was subtracted from each pixel value, so that only the change in pixel brightness was used to calculate the applied pressure.

Data collection and manipulation:

Thumb pressure was exerted on the plate to produce a series of images which were filmed, and the simultaneous voltage traces produced by the load cells were recorded. The video was run through the computer, and a series of frames captured and time-stamped by the frame grabber. The images were then analysed, having set the threshold, and measured the background levels. The area and mean light intensity (in pixels) of each image was measured, and the results put into an excel spreadsheet. The background level was then subtracted from each pixel value, and the results stored along with the frame number and time-stamp.

In the second part of the process, the relevant trace from the oscilloscope was identified and analysed. The software package (Virtual Bench) produces a report of each trace, as a text file, and this can be exported into the excel spreadsheet containing the image data. The Virtual Bench report contains the actual values from both traces, along with the time points at which they were sampled (usually 0.002 second intervals). The time at which the leading edge of the square wave 'drops' was identified, and the simultaneous point on the load cell voltage trace was taken as time zero (see Figure 2.2).

The first frame showing the light bulb image was found, and the time-stamp noted,

and this was taken as time zero. Each frame after that has the initial time subtracted, giving it a time relative to this start point. The voltage readings matching these time points were then located in the report, to give the voltage output produced by the force being applied to create that particular image. It was accepted that there was a degree of error introduced by the fact that the frames were captured from the video at intervals, rather than continuously. If, for example, the light bulb actually switched on a few milliseconds before the frame on which it first appeared was captured, then the voltage reading assigned to it will be incorrect. The error is likely to be small, however, as the interval between frames was so short (usually less than 0.03 sec), during which time, the voltage readings rarely changed significantly. The voltages were then converted into force values using the previously established formula (mass = ('voltage'+ 4.979)/0.547). Knowing the areas over which the forces were exerted, it was then possible to calculate the pressures.

The pressures were then plotted against the change in pixel value (= 'light intensity'), as a scattergraph using Cricket Graph software. A curve could then be fitted, and the appropriate equation obtained, along with the r and r^2 values. This method was used to produce calibration curves to test the system. For the actual data analysis in the first experiment (Chapter 3), the calibrate function in Image was used: as Image can only use a maximum of 20 data points, the 100 values used in the calibration curve were reduced by meaning every 5 in sequence, to reduce the number of points to fit into the Image software.

Two problems became apparent during the initial calibration tests:

Test 1:

A series of 20 thumb prints were created by the application of a range of pressures, and the data showed a good correlation between pressure and change in pixel value, with r values averaging 0.95. When the actual points were plotted on a graph, however, the slopes of the lines were quite variable. The six 'high pressure' tests were all similar, as the pixel intensity was fairly even across the area, particularly when the surface area remained similar. Problems became apparent at lower pressures, however, when pixel intensity and area showed large variations. These plots showed much higher changes in pixel value for much lower pressures, and it was thought that they could have been created by applying high pressure, and then taking measurements as the pressure was removed. This would facilitate the errors previously discussed: the lag effect, where the unloading curve is slower, and a possible creep effect, if the application of pressure had been prolonged at the start. However, the effects seemed too extreme to be caused by this alone.

Further consideration of the aberrant trials showed that they appeared to occur in pairs or clusters, suggesting that the problem was not random. The following possibilities were considered:

a) that the frames were slightly out of synchronisation with the voltages. The pixel values were then plotted against the voltages all moved 'forward' two, and all moved 'back' two, but the slope of the line changed only very slightly.

b) that the plate may be 'sticking' i.e. not moving down freely under the applied force, so that a greater force (producing a greater change in pixel value) would deflect the strain gauges to a small degree (registering a lower voltage).

The equipment was partially dismantled and reconstructed to ensure free movement of the plate, and this seemed to solve the problem. The weight calibration was rechecked, and is illustrated in Figure 2.4. It was still found to be linear ($r^2 = 1$), but now described by the equation: mass = ('voltage' + 1.123) / 0.549, suggesting that the plate may indeed have been restricted from moving freely.

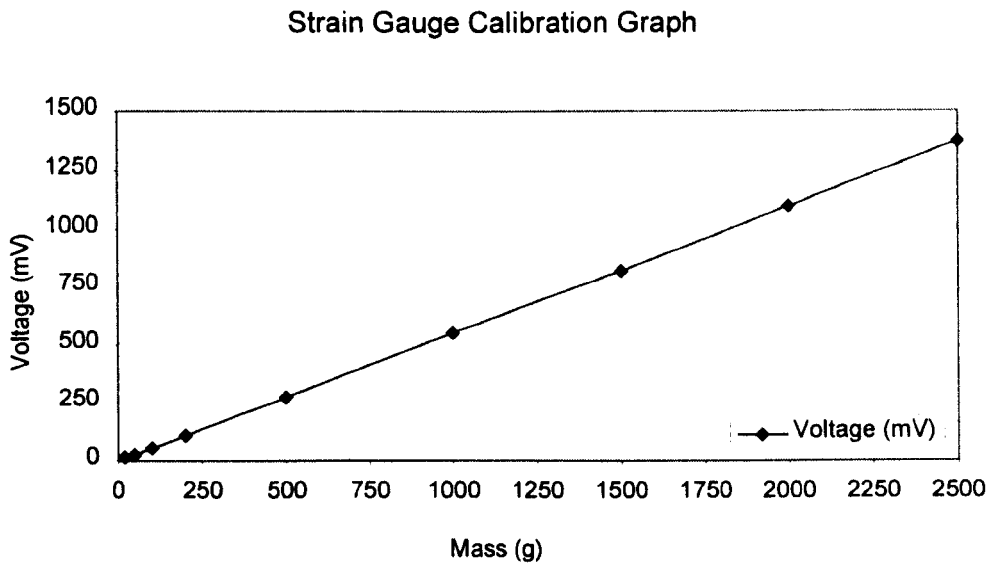


Figure 2.4: Calibration graph illustrating strain gauge voltage output for applied mass.

Test 2:

Thumbprints were again used to produce 10 light image movies for analysis, and the slope of these graphs were found to be well aligned, with similar r values as before. On closer examination of the graphs however, a second problem became apparent. In several of the plots, the initial value was abnormal, showing a much higher pixel intensity for the force that apparently produced it. It is possible that these images were captured as the light bulb (used for synchronisation) was switched off, and so residual light from the bulb may have affected the image. The first value in each calibration test was therefore discounted, but this problem will not occur when testing birds, as the equipment will be on and running before the birds cross the plate, and no synchronisation light will be used.

Results:

An example of a calibration curve produced for the system is illustrated in Figure 2:5.

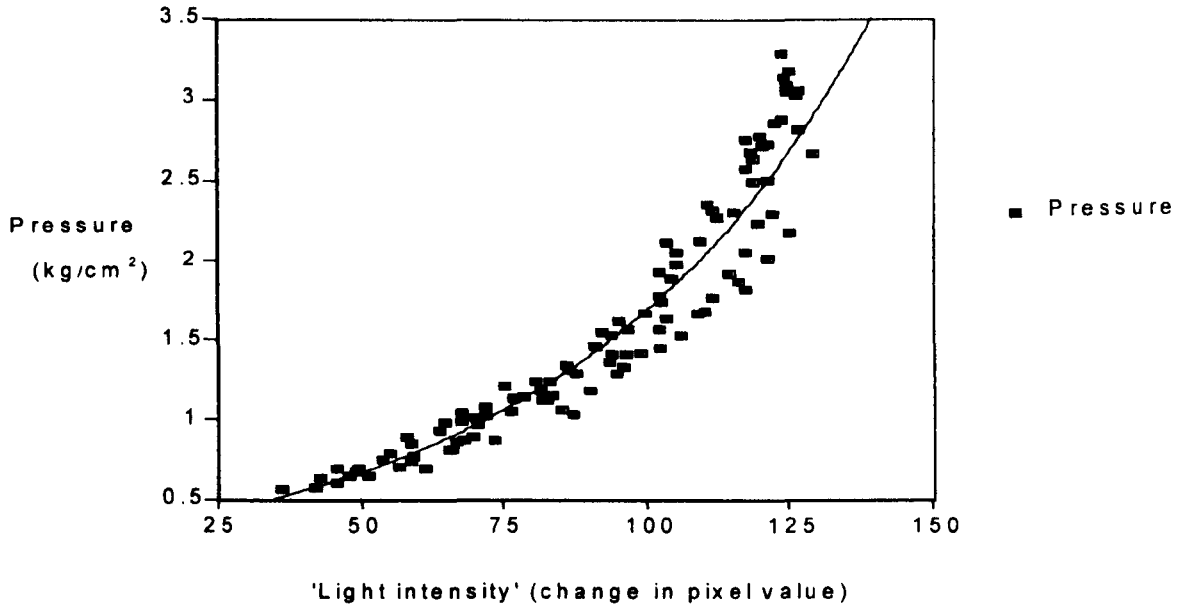


Figure 2.5: Calibration curve showing the relationship between applied pressure and change in pixel value ('light intensity).

The data was plotted using Cricket Graph software, and the relationship between 'light intensity' (change in pixel value) and pressure is described by an exponential function: $\mathbf{y = 0.295 \times 10^{0.008x}}$, where y = pressure, and x = light intensity (r = 0.973, r2 = 0.947).The r value gives an indication of the relationship between the x and y variables, without implying causation. The r2 value is more useful, as it indicates the spread around the mean value e.g. plotting the known pixel value on the X-axis, and the unknown (pressure) on the Y axis, an r^2 value of 0.95 suggests that there is a 5% variability around the mean pressure calculated from a measured pixel value. This can be thought of in terms of error: 5% is a very small percentage error, and is acceptable for this dynamic system, with so many potential sources of error.

It can be seen from Figure 2.5 that, unlike the original system described by Betts *et al* (1980 a,b,c,d), the present system does not show a linear relationship between pressure and light output over the whole range of calibration. The values in the middle of the range approached linearity, but deviate from this at each end of the range. It is possible that the aforementioned authors may have restricted their 'working range' to this linear portion of the graph. If the equation proposed by Betts *et al* (1908d) is considered:

total force = Calibration Factor (CF) x total active area x total light,

it can be re-arranged by dividing each side by 'area' to produce the equation:

pressure = CF x total light

which is the basic form of the equation:

$\mathbf{pressure = 0.295 \times 10^{0.008x}}$,

where 0.295 is the calibration factor, and x = light intensity.

It is important to remember that light intensity is a representation of pressure, not an actual pressure value. The light intensity must be multiplied by a calibration factor to convert it into units of pressure (e.g. kg/cm^2). This takes into account the absolute effect of the pressure producing the light intensity in each pixel over the whole area. It should also be noted that the present system appears to 'saturate' at a pressure of approximately $2.5kg/cm^2$, which produces a change in pixel value of around 125. Values above this can therefore only be described as "greater than 2.5 kg/cm^2 ".

2 : 2 KISTLER FORCE PLATE

Technical details:

The Kistler force plate (KFP) is designed with four 3-component force transducers fitted between a base frame and a top plate. Each 3 component force transducer contains 2 pairs of shear-sensitive quartz crystals for Fx and Fy forces, and one pair of pressure-sensitive quartzes for Fz forces.

The force plate is connected to an 8-channel charge amplifier and junction box (Types 5865C and 5606, respectively), where the electrical charges yielded from the force transducers are converted into electrical voltages. An analogue to digital converter (ADC) board is installed in the computer (Dell 433s/L), which multiplexes and converts the analogue signals from the charge amplifier into digital signals for processing and plotting by the Bioware software (version 2.0). This enables the calculation of the numerous gait parameters, including: 3 ground reaction forces (Fx, Fy and Fz), three moments of force (Mx, My, Mz), torque about the vertical axis (Tz), total force (F), total moment (M) and the coefficient of friction. All of the aforementioned information is stored automatically, however the present study was restricted to analysing the 3 ground reaction forces.

Testing set-up:

The force plate was set up in the testing room, with the runway built around it (see Figure 2.6).

The force plate must be set on level ground, and firm at all points of contact with the floor. The floor itself must be solid, as any vibrations from either the plate rocking slightly, or a person stepping next to it, can trigger it prematurely. A rubber mat was placed below the forceplate to absorb vibrations, and the wooden cover screwed firmly to the top plate. Although the plate itself often becomes soiled, the cables, charge amplifier and computer must be protected from dust. The charge amplifier was given a 30 minute warm up period and left on throughout the testing period (even when the equipment was not in use) to maintain stability.

Figure 2.6: Kistler Force Plate testing set-up.

Two CCD cameras were used, one positioned directly above the forceplate (Polnix TM 526A with a COMISAR 4.9mm lens), and one lateral to the plate i.e. perpendicular to the direction of travel (Polnix TM 528A with a COMISAR 8.5mm lens). The dorsal video was spatially calibrated by placing a grid of known dimensions in its field of view, however the data obtained from this perspective has not been included in this thesis. The side of the runway was made of wide bore straight mesh, which provided a useful mechanism for spatially calibrating the lateral video, and a millisecond timer was placed in view of the camera to allow accurate calculation of speeds. The charge amplifier connected to the forceplate was positioned in the field of view of the lateral camera. This had two lights, one of which came on when data acquisition was started manually, the other when force was applied to the plate, making synchronisation of the force records and video simple. The camera signals were recorded on Panasonic video recorders (AG67720), which also placed a time stamp on the films. Initially the videos were analysed to calculate speeds and cadences, and give information on direction of individual footsteps. They could also be

used in future to analyse other aspects of gait, such as lateral sway, and relate it to the GRF data.

The plate was calibrated during manufacture, and the calibration can be checked simply by standing on the plate, as the vertical force should equal the weight of the subject, independent of where the load is applied to the plate surface. The sensitivity range was altered to one suitable for chickens, anticipating the maximum load as being 10 x weight of animal. Extending the range much further causes a compromise in sensitivity, especially at the higher values.

Acquisition settings:

A sampling rate (frequency response) of 100 Hz was chosen so that the highest frequency components of the GRF were recorded e.g. stride period of 100ms = 10 Hz, so the plate frequency has to be at least 100 Hz. This follows the standard recommendation that the 'platforms natural frequency response should be of an order of magnitude (x 10) higher than the primary signal frequency' (Biewener and Full, 1992). The sampling period depends on the average walking speed over the plate: for the Brown Leghorns, a 2 second period was sufficient, but this was extended to 6 seconds for the slower walking broilers, and up to 10 seconds for lame birds.

The forceplate can be set to record either on a keystroke, or triggered automatically, when load above a predetermined level is applied to the plate. Although automatic triggering is normally used (to make synchronisation easier), the trigger sensitivity was set so low for the birds (at 0.1kg), that it often triggered prematurely, and so was set to manual instead. The direction of data acquisition must also be set, determined by the direction in which the subject crosses the plate on each run. This is the basis on which the positive and negative signs are allocated to the data values e.g. if lateral force is given a negative sign and medial force a positive sign, the software will need to be told in which direction the subject was moving across the plate in order to distinguish these two components, and allocate the signs correctly between different runs. It can be set to autoswitch, so that the recording direction alternates between each run.

Figure 2.7 illustrates the plate and conventions used throughout this thesis.

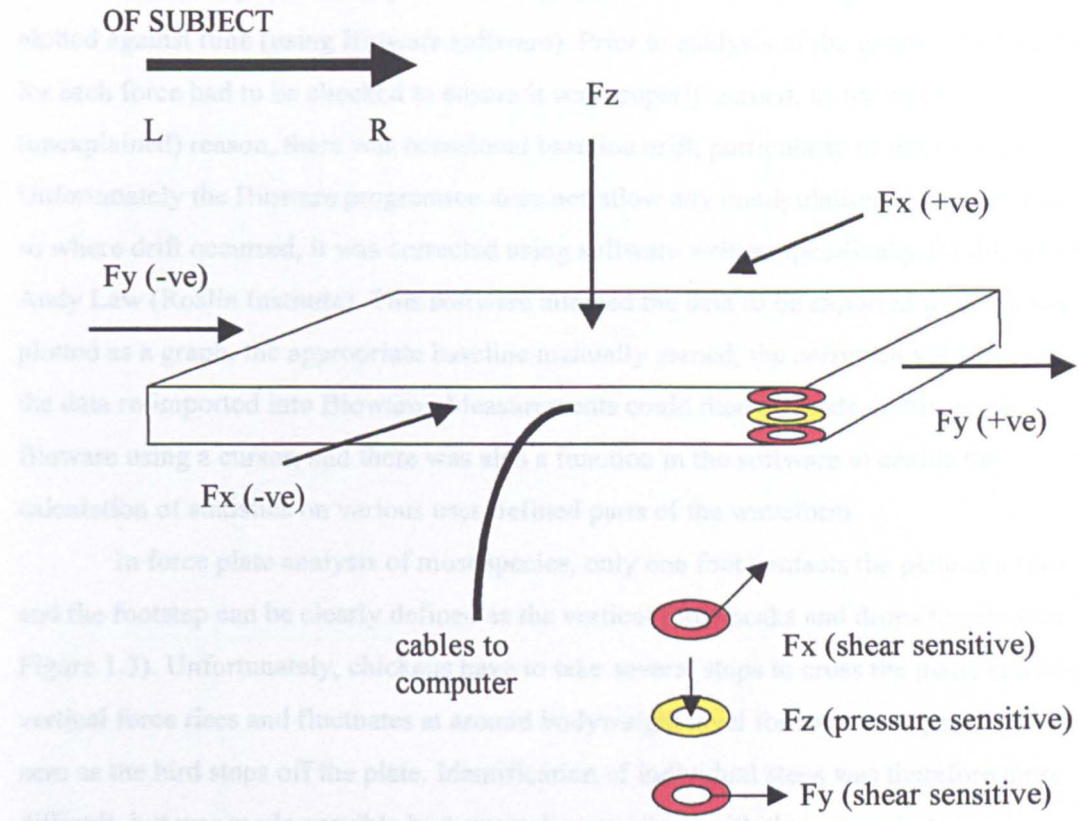


Figure 2.7: Kistler Force Plate Conventions.

Facing the lateral aspect of the runway (on the side with the cables), the end to the right side was designated positive, and to the left, negative. Fx is the force along the short force plate axis (or 'side-to-side' force), and Fy the force along the long force plate axis (or 'braking and propulsive force). Unfortunately, these conventions are not standard between all force measuring systems. When measuring the three ground reaction forces, some 'cross-talk' occurs between the three channels, which is estimated as being <1% by Kistler (up to 3% is acceptable; Biewener and Full, 1992)

Data Handling:

Each run is presented graphically on the computer screen within seconds of being collected. Accepting the run causes it to be saved as a .dat file, with the number of the run also being saved e.g. May001.dat. Simultaneous with the force plate data collection, video films of the runs were made, and time-stamped. Recording the run number and timing allowed the video and forceplate records to be synchronised for later analysis, to calculate speeds and cadences, and to identify the events that produce the forces.

The force plate data is presented in graphical form, with the ground reaction forces plotted against time (using Bioware software). Prior to analysis of the graphs, the baseline for each force had to be checked to ensure it was properly zeroed, as for some (unexplained) reason, there was occasional baseline drift, particularly of the Fz trace. Unfortunately the Bioware programme does not allow any manipulation of the raw data, and so where drift occurred, it was corrected using software written specifically for this by Dr Andy Law (Roslin Institute). This software allowed the data to be exported from Bioware, plotted as a graph, the appropriate baseline manually zeroed, the corrected values saved, and the data re-imported into Bioware. Measurements could then be made on the graphs in Bioware using a cursor, and there was also a function in the software to enable the calculation of statistics on various user-defined parts of the waveform.

In force plate analysis of most species, only one foot contacts the plate at a time, and the footstep can be clearly defined as the vertical force peaks and drops to zero (see Figure 1.3). Unfortunately, chickens have to take several steps to cross the plate, and so the vertical force rises and fluctuates at around bodyweight level for several steps, returning to zero as the bird steps off the plate. Identification of individual steps was therefore more difficult, but was made possible by temporal comparison with the concurrent braking/propulsion force graph, as this trace is produced only by the foot that is in ground contact (although influenced by the swing phase of the other one) (Rumph *et al*, 1993).

A typical graph produced as a bird walked across the plate is illustrated in Figure 2.8, which is annotated to show the measurements that were made. This should be consulted with section 2.3.

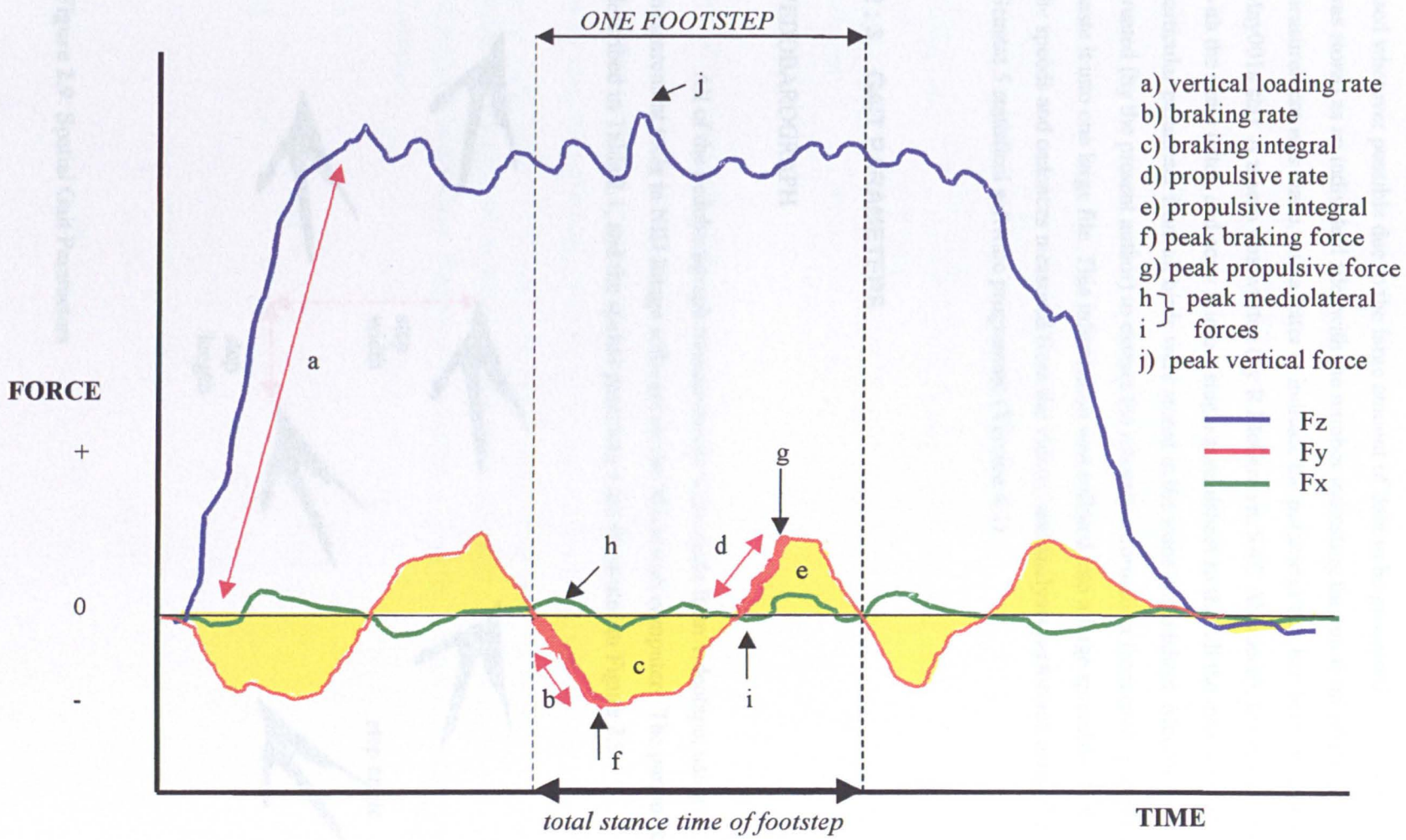


Figure 2.8 : 'Typical' ground reaction force trace of a walking chicken, annotated to show various parameters.

Each measurement had to be made manually from the graphs, but macros were then used wherever possible due to the large amount of data to be processed. Each measurement was stored as an individual file, with the number indicating the run from which the measurement was made, and a letter to indicate the parameter that had been measured (e.g. May001a.sta). A macro was written (by R.McGovern, SAC Aberdeen), to open each file with the same letter, and copy it into a single spreadsheet so that all the measurements for a particular parameter from all birds were stored in the same spreadsheet. Macros were then created (by the present author) to extract the relevant information from each spreadsheet and paste it into one large file. This information was collated into a large spreadsheet, along with the speeds and cadences measured from the videos, and analysis performed using the Genstat 5 statistical software programme (Version 4.1).

2 : 3 GAIT PARAMETERS

PEDOBAROGRAPH

All of the pedobarograph measurements were made from videotape, using measurement tools in NIH Image software on the Macintosh computer. The parameters are described in Table 2.1, and the spatial parameters are illustrated in Figure 2.9.

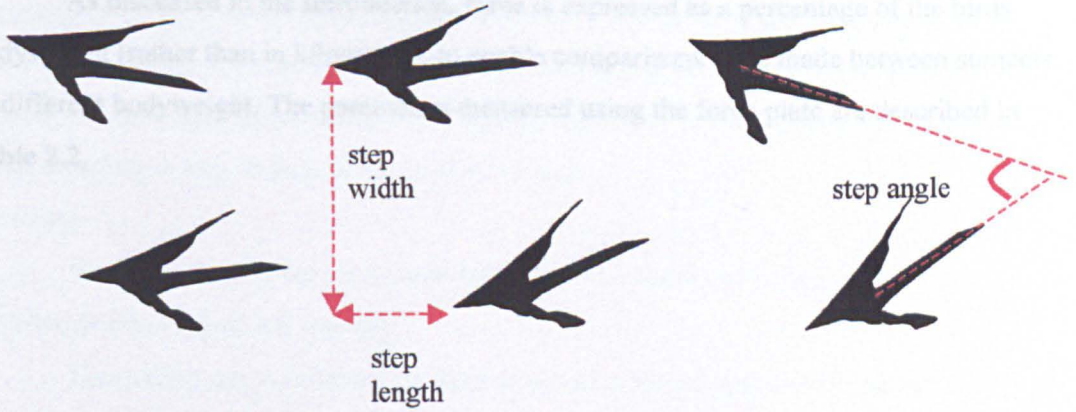


Figure 2.9: Spatial Gait Parameters

Table 2.1: Gait parameters measured using pedobarograph.

Speed (m/sec)	calculated by dividing the distance the bird moved across the plate by the time interval between the first and last point of foot contact
Cadence (steps/min)	or step frequency, is the number of steps taken in a given time
Step Length (m)	the distance between corresponding points on successive footprints of opposite feet
Step Width (m)	or walking base, is the lateral distance between placement of the two feet, measured between the two metatarsal pads
Step Angle (degrees)	the angle of placement of the two feet on the ground, measured at the point at which a line drawn along the midline of each middle toe would meet.
Plantar Pressure (kN/m^2)	the pressure created at the foot-recording surface interface (which is a function of the force divided by the area over which it is distributed). (1 kg/cm^2 = 98.1 kN/m^2)
Stance time (s)	the period when a particular foot is in ground contact during one complete gait cycle
Swing time (s)	the period when a particular foot is raised off the ground during one complete gait cycle
Double contact time (s)	the period when both feet are in contact with the ground at the same time during one complete gait cycle.

KISTLER FORCE PLATE (refer to Figure 2.8)

The force measurements were made from the waveforms produced by the Bioware software, from data collected by the forceplate. The speed and cadence measurements were made from the videotapes, which were obtained synchronously with the force records.

As discussed in the Introduction, force is expressed as a percentage of the birds bodyweight (rather than in kilograms), to enable comparisons to be made between subjects of different bodyweight. The parameters measured using the force plate are described in Table 2.2.

Table 2.2: Gait parameters measured using Kistler Force Plate.

Vertical force (%bw)	the largest of the three ground reaction forces
Horizontal force (%bw)	separated into braking (negative) and propulsive (positive) forces.
Mediolateral force (%bw)	separated into medial and lateral forces
Impulse (bw.s)	the area under the force-time curve, representing total load over time (also known as the integral).
Slope (bw/s)	the rate of change of the force
Stance time of a run (s)	the total time taken for a bird to cross the plate, measured from the point at which the vertical force trace rises from zero as the bird steps onto the plate, until it returns to zero as the bird steps off the plate.
Stance time of a step (s)	the time from first contact of a foot until the same foot is lifted from the plate, measured from the craniocaudal force trace.
Speed (m/sec)	measured directly from the videotape, calculated by dividing the distance the bird moved across the plate by the time interval between first and last point of foot contact
Cadence (steps/min)	the number of steps taken in a given time, measured from the videotape.

2 : 4 BIRDS

Three types of birds were used in this work:

Brown Leghorns:

These are similar in general body shape and size to the 'ancestral' jungle fowl. They were originally bred as an egg laying strain, but have had no selection pressure since the late 1960's, and have a normally functioning locomotor system. They are used in this work to establish some normal values for the chicken, against which to compare the broilers. A small breeding colony is kept at the Roslin Institute.

Broilers:

Broilers are the meat production birds, and two types were used in this work:

Relaxed broilers (Ross AK strain):

These birds are maintained by Ross as a random-bred population, mainly for genetic controls. Predecessors of the modern broiler, they have had no selection pressures on them since approximately 1972. They are normally fed *ad libitum*, and have few musculoskeletal problems.

Selected broilers:

These were Ross 308 strain selected birds, the most recent high production meat bird produced by the Ross Breeding Company. They are 25 generations on from the relaxed

bird, and although selection for high growth rates and feed conversion efficiency is still the primary concern, efforts have been made over the last few years to select for improved leg strength. The conditions under which the birds were reared and kept were as close as possible to commercial conditions, as described in the Ross Breeders Management Manual (1996), and summarised in Appendix 2.1.

Feeding:

Two basic feeding regimes were used. Some of the groups were fed *ad libitum*, that is, having free access to food at all times, and others were restricted-fed. Broilers are not normally restricted-fed, and so the commercial restriction ration used by Ross (Ross Breeders, 1995) in rearing their broiler breeders (parent stock) was applied (Appendix 2.2). A modified restricted feeding regime was applied to a group in one of the experiments, and this is described in the relevant section (Chapter 4).

Training:

Little was done in terms of training the birds - rather they were allowed to familiarise themselves with the surroundings and the equipment, and move of their own 'free-will', at their own 'preferred walking speed' (Steven *et al*, 1983). The individual testing set-ups will be described in the appropriate experiments.

Training birds to walk in a 'predictable' manner has rarely been successful, and it was felt that it might alter their normal locomotion e.g. locomotion on a treadmill can be abnormal as the subjects control their speed to match the rate of the belt (Wetzel *et al*, 1975). Familiarisation with the testing environment was very important, however, as exposure to a novel environment, even if it is not frightening, can cause birds to alter their gait (Gentle and Corr, 1995). It was decided not to use food as a reward for walking, due to the very different levels of motivation this would create in the birds on different feeding regimes - the *ad libitum-fed* birds being reasonably satiated, compared to the restricted-fed birds, which were ravenously hungry and would run to food. Aside from changing the gait, it was also felt this would mask any lameness. Nor was it considered appropriate to 'threaten' the birds to encourage them to move, as apart from the welfare aspects of this, Gentle (1993) has shown that nociceptive stimulation results in either escape responses or crouching by the bird, which would obviously be of little use. It was clear from early pilot trials that the birds tended to stop and turn to face anything perceived as unusual or threatening, rather than to move away.

In the pedobarograph experiment, a group of birds were placed at one end of the

runway, and one bird at a time was taken to the other end, and let walk back down the runway to join its companions. This worked extremely well, especially if the person moving the birds remained at the other end. In the force plate work, the birds' home pen was placed at one end of the runway, and the birds walked back to that. With these approaches, it was assumed that the motivation of each bird would be similar.

2 : 5 STATISTICAL ANALYSIS

A population is rarely entirely uniform, and therefore certain precautions are taken when using samples to draw inferences about the larger population. The natural variation occurring within a population is compounded by variation arising from other sources, which may or may not be controllable. It is possible to avoid introducing systematic error (e.g. through the effect of elapsed time or order of testing), observer bias, and imprecision into the results, but there will always be a degree of measurement error (e.g. variation in equipment), random error and sampling error. To ensure that the particular 'samples' used in this work were as representative of the populations as possible, the following methods were used.

Randomisation

The chickens were randomly allocated into groups, and treatments were also randomly allocated to animals within a group, so that each had an equal probability of being allocated a particular treatment. This decreases the likelihood of any systematic error occurring, so that any treatment effect will then be only subject to random variations.

During testing, more limited randomisation was practised: small sets of birds were chosen at random and run in groups, until half their required runs were collected. All the birds were tested in the morning, then randomly re-allocated into different small groups for testing in the afternoon, to collect the remaining runs. In the same way, data was analysed randomly, so that systematic error e.g. through fatigue or boredom did not arise. It is accepted that ideally, each bird should have been selected randomly, tested once, then replaced, and another chosen until 10 runs were obtained from each. However this would have prolonged each testing period into a second day, which could introduce greater likelihood of systematic error.

Replication

To determine how many birds should be used, it would be necessary to have some idea of how much variation exists in the population, and how big a treatment effect is likely to be significant. While a bigger sample is obviously more likely to be representative, there is a point at which increasing size will bring no improvement in accuracy, and so become wasteful. Unfortunately it is often not possible to determine this without a pilot trial, and factors such as time, space and cost are often given equal weight.

Where possible, the same number of birds were allocated to each group, to 'balance' the data and make the analysis easier, however the number of runs obtained was very much dependent on the willingness of the bird to walk with minimal encouragement. The birds were usually willing to give between 5-10 runs each without much encouragement, and although the data set could have been increased by increasing the number of runs taken, attempting to get more could cause changes in the variance within birds (due to fatigue, habituation or frustration, perhaps, of the observer as well as the bird). An average of 7 'acceptable' runs per bird was therefore aimed at, where an 'acceptable' run was defined as one with a fairly straight path, at a reasonably constant speed. This rationale is an accepted method to introduce some constancy to the gait pattern under study (Leach and Cymbaluk, 1986; Budsberg *et al*, 1987; McLaughlin and Roush, 1994).

The number of steps taken by the birds varied between 1 and 4 per run, and was largely determined by step length, and therefore ultimately limited by the length of the plate. As step number increased with decreasing step length, due to the fixed length of the plate, using all the steps from every run would result in an over-representation of short step lengths in the data. It was therefore decided to use only one step from each run, to avoid introducing bias in the means and variances in those measurements made on an individual step (length, width and angle). This step was chosen at random from each run.

Distribution (or 'normality' of shape of the population):

The 'normality' of a sample can be assessed by looking at the distribution of the data points when plotted on a graph. A normal distribution appears as a 'bell-shaped' curve, as this arises when the measurements are of objects which are fairly similar, so that the variation that exists is due to a number of different factors exerting only a small random influence (either positive or negative) (Rees, 1989). This enables certain inferences to be drawn about the population from the sample. If there were a few very large or small values, this would 'skew' the curves in one direction or the other. In such a case, it is usual to 'transform' the data in one of several ways, to normalise it, and also stabilise the variance.

The commonest way is the logarithmic transformation, where the data values are converted to their logs prior to analysis. Most of the measurements made in this study were not normally distributed, and were transformed to logarithms before analysis. This would be expected for such measurements which could theoretically extend to infinity on the right-hand side of a graph, but are limited by zero on the left-hand side. Where there were some zero values e.g. for step width and step angle, (and negative values were theoretically possible if steps crossed over or toes pointed out), these values were left untransformed. Percentage values and ratios were also not transformed. The way the data has been analysed is described in each experiment.

Where data has been transformed, the median is given as a more accurate estimation of the average, (otherwise, means are used). To give an indication of how variable the medians were, first order approximation was used to produce a standard error of the median, calculated from (median x standard error of the log median) (Kendall and Stuart, 1963).

Variability

In theory, if the population does not vary too much, then there is a good chance that a sample will be representative. There were 4 main sources of variation to consider in these experiments: the variation between the birds, the variation within a bird (both between runs, and within runs between steps), and finally, the inter-sessional variability.

The variance of a gait measurement about the population mean can therefore be expressed as the sum of its components, the between session, between bird and within bird components of variance. It is useful to know the proportion of the variance arising from each, and so the population means and the components of variance were estimated by residual maximum likelihood (REML), (Patterson and Thompson, 1971).

Accuracy of results:

As sample means are not 100% accurate representations of the population means, it is necessary to give an indication of how good an estimate they are thought to be, known as a 'confidence interval'. It is usual to choose a confidence interval of 95%, which means that the stated result is likely to be correct 19 times out of 20 (the one 'miss' being due to sampling error). The 95% ranges are therefore given, to indicate the range within which a measurement of a variable from any bird taken at random from the general population should fall. These will obviously be large if the variance is great.

Relationship between variables:

A final consideration is the independence of the variables. Where one or more variables may be dependent or influence each other, it is important to consider this in analysing the data. The two main variables we need to consider in this way are speed and bodyweight.

The mass of the body under gravity is equivalent to weight, which produces the force acting vertically downwards. Thus a heavier animal will exert a greater force, irrespective of any treatment effect. It is therefore standard practice to 'normalise' the data by dividing by the subjects own bodyweight (bw) and expressing the results as a % bw, which allows comparisons to be made between subjects of different weights. The rate of change of force (the slope of the line), and total force over time (integral) are also divided by bodyweight, and expressed as bw/s, and bw.s respectively.

Speed is a function of distance x time, and is dependent on step length and step frequency. As it was not possible to control the speed of the chickens, the dependence of gait measurements on speed and step length was investigated. This was done by regression on the logarithm of speed or length again using REML to estimate the variance components and the means and regression coefficients. The data was also analysed within speed ranges, to minimise any confounding effect of speed. The ranges were defined as follows (McLaughlin and Roush, 1995):

'medium' speed = mean ± ½ SD

'fast' speed = ¼ SD above 'medium' speed, spanning 1 SD

'slow' speed = ¼ SD below 'medium' speed, spanning 1 SD

The following ranges, obtained using the data from the Brown Leghorns in Chapter 3, were used in all the subsequent experiments:

'fast' speed range = 0.793 – 1.049 m/s

'medium' speed range = 0.473 – 0.729 m/s

'slow' speed range = 0.153 – 0.409 m/s

An alternative method would have been to establish separate ranges for broilers, on the basis that they probably walked more slowly overall i.e. a broiler moving in the 'medium' speed range for a Brown Leghorn may in fact be a 'fast' broiler. However, it was decided to make comparisons within a single set of ranges, as gait parameters should depend on absolute speed, irrespective of bird type.

CHAPTER 3:

A STUDY OF BROWN LEGHORN GAIT, USING THE PEDOBAROGRAPH.

3. 1 INTRODUCTION

This first experiment was designed to test the pedobarograph as a system for gait analysis of poultry, and to establish a range of spatial and pressure values to objectively describe walking in normal birds. Due to the lack of control over the subject's speed, it was expected that the variability in the results would be high, and so some time was spent investigating the most appropriate methods for analysing the data.

3. 2 MATERIALS AND METHODS

Birds

Twelve adult Brown Leghorn hens, a laying strain with a low incidence of leg problems, were used in the study. The birds were 29 weeks old, with an average weight of 1450g (+/- 100g). They were kept as a single group on a deep-litter floor system for 8 weeks, and familiarised with the runway in the week before testing. All the birds were normal on clinical examination, and showed no sign of lameness.

Equipment and calibration

The pedobarograph is described and illustrated in Chapter 2.1 (General Materials and Methods). In this experiment, the 'calibrate' function in Image was used to produce a calibration curve relating applied pressure to mean light intensity, described by the exponential equation $y = 0.295 \times 10^{0.008x}$, where y = pressure ($kg/cm^2$), and x = light intensity (pixels), with an r^2 value of 0.992. The curve is illustrated in Figure 3.1.

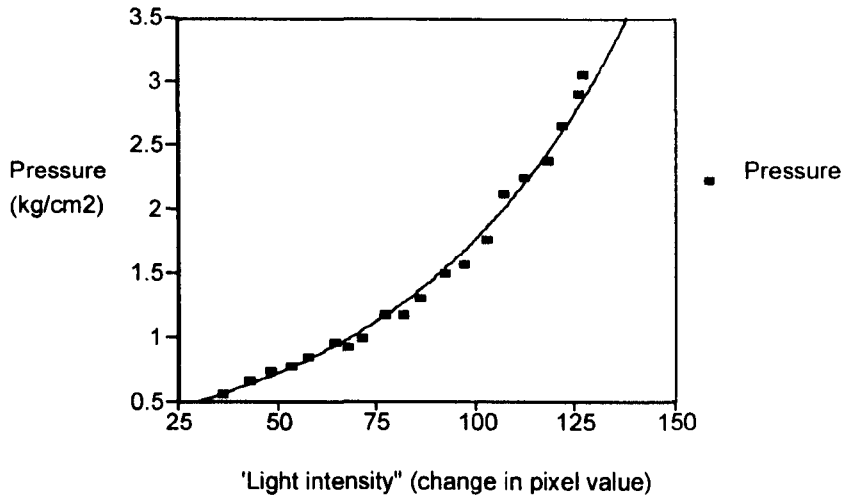


Figure 3.1: Calibration curve using meaned values to relate pressure to light intensity.

As explained in the materials and methods section, Image can only perform a calibration based on a maximum of 20 paired values. It was thought to be more accurate to take 100 paired measurements, and reduce them down to 20 by meaning every 5 (sequentially). Thus the equation presented here was generated from the data points presented in Figure 2.5.

Testing procedure:

The birds were tested in groups of 3: each bird in turn was placed at the opposite end to the other 2, and allowed to walk back towards them, stimulated by the observer standing slightly behind them and moving forwards. An average of 15 runs was collected from each bird, to produce between 5 and 9 runs per bird which were considered suitable for analysis. Only those runs in which the bird took an approximately straight path across the plate at a reasonably constant speed were analysed. Runs where the bird paused or deviated markedly in direction were discounted. Five runs from each of six birds of a similar weight were used to investigate plantar pressures.

Gait Parameters *(see Table 2.1)*

In this initial experiment, the plantar pressures under the foot were measured, along with the spatial parameters of step length, step width, step angle. Speed and cadence were also calculated.

Statistical Methods (*see Chapter 2.5*)

A single step was chosen at random for analysis from each run. Speed, cadence and length were transformed to logarithms before analysis: width and angle were left untransformed as there were some zero values, and negative values were theoretically possible.

Means and 95% confidence intervals of the untransformed measurements were derived from the mean and variance estimates in the logarithmic scale using properties of the lognormal distribution (Hastings and Peacock, 1974). The population means and the components of variance were estimated by residual maximum likelihood (REML) (Patterson and Thompson 1971). For length, width and angle, the between steps-within run variance was estimated on the full data set by pooling sample variances within runs.

The dependence of gait measurements on speed or length was investigated by regression on the logarithm of speed or length, again using REML to estimate the variance components and the means and regression coefficients.

The maximum pressures measured at each point were averaged for the 5 runs for each bird, as were the forces that were subsequently calculated.

3. 3 RESULTS

Table 3.1: Spatial Gait Parameters: Coefficients of variation, means and 95% confidence intervals.

CV (%) of single observations from:	Measurement ‡				
	Speed* (m/sec)	**Cadence*** (steps/min)**	**Length *ns* (m)**	**Width *ns* (m)**	**Angle*** (degrees)**
different birds	42	33	23	54	41
different runs within a bird	37	27	22	51	31
different steps within a run	-	-	24	60	31
population mean	0.10	53.5	0.11	0.02	33.3
95% confidence interval of mean	0.09-0.10	46.8-61.2	0.106-0.122	0.018-0.025	27.1-39.4
bird overlap •	92	83	96	95	74

‡ Statistical significance of bird component of variance (in logarithmic scale where appropriate) at *** $p < 0.001$ * $p < 0.05$ *ns* $p > 0.1$

• The average percentage overlap of the 95% ranges of large samples of observations from 2 birds chosen at random.

The coefficients of variation, means and 95% confidence intervals for the spatial gait parameters are shown in Table 3.1. The variability between birds is high for all parameters. The variability within a bird is only slightly less: there is equal or greater variability between steps within a run as there is between the runs made by an individual bird.

Table 3.2: Spatial Gait Parameters: 95% ranges for a newly observed bird mean.

Number of runs	Measurement				
	Speed (m/sec)	**Cadence (steps/min)**	**Length (m)**	**Width (m)**	**Angle (degrees)**
5	0.06 – 0.17	32 - 87	0.09 – 0.15	0.01 – 0.04	11 – 56
7 *	**0.06 – 0.17**	**33 - 85**	**0.09 – 0.15**	**0.01 – 0.03**	**11 – 55**
10	0.06 – 0.16	33 - 84	0.09 – 0.14	0.01 – 0.03	12 – 55
15	0.06 – 0.16	34 - 83	0.09 – 0.14	0.01 – 0.03	12 - 54

* average number of runs used in present study

Table 3.2 illustrates the range within which 95% of bird means would fall. The ranges for each measurement are large, as would be expected given the coefficients of variation (C'sV) shown in Table 3.1. The results also show that increasing the number of runs performed would not greatly narrow the range, even if more than double the number of runs used in the present study were completed.

Table 3.3: Spatial Gait Parameters: Relationships between measurements

Regression on (log) SPEED	**Mean**	**SE**	**Regression coefficient**	**SE**
Cadence (log)	3.94	0.03	0.66 ***	0.0391
Length (log)	2.41	0.03	0.33 ***	0.0530
Width	2.13	0.18	- 0.44 ns	0.3180
Angle	33.3	2.81	0.99 ns	3.2610
Regression on (log) LENGTH	**Mean**	**SE**	**Regression coefficient**	**SE**
Width	2.14	0.16	- 0.49 ns	0.5527
Angle	33.3	2.84	6.13 ns	0.5710

Regression coefficient statistically significantly different from zero: *** $p < 0.001$
ns $p > 0.1$

The relationship between the different spatial parameters is shown in Table 3.3. Cadence and step length both increased with speed. The results indicate that step width and step angle, however, are not significantly influenced by speed, or step length.

Pressure is not distributed evenly across the plantar surface of the foot during walking, but concentrated in the areas of the digital pads, as illustrated in Figures 2.3 and 3.2.

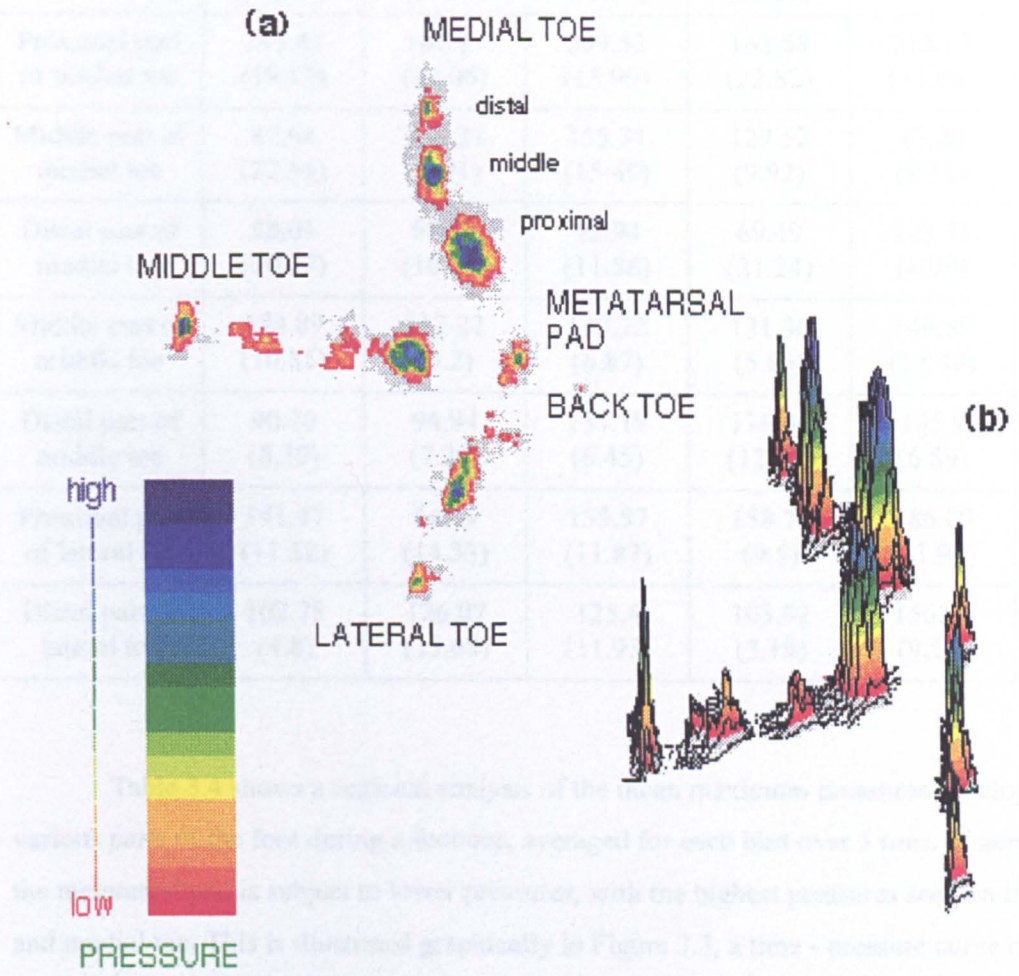


Figure 2.3: a) Computer-generated pseudocolour image of footprint, 'captured' from pedobarograph.
b) Computer-generated surface plot of footprint.

Table 3.4: Regional analysis of mean maximum pressures under foot, in kN / m^2 (±SEM).

	Bird 1	**Bird 2**	**Bird 3**	**Bird 4**	**Bird 5**	**Bird 6**
Back toe	182.56 (17.52)	195.52 (5.6)	146.1 (5.31)	183.24 (11.19)	143.06 (12.51)	166.61 (13.98)
Metatarsal pad	51.76 (13.49)	131.17 (17.64)	38.47 (16.47)	16.32 (16.32)	88.42 (11.91)	96.35 (13.61)
Proximal part of medial toe	149.41 (19.13)	186.87 (22.06)	209.52 (15.99)	162.88 (22.82)	218.03 (11.06)	190.3 (23.92)
Middle part of medial toe	87.64 (22.54)	113.24 (6.91)	155.31 (15.49)	129.52 (9.92)	43.80 (8.52)	141.34 (22.95)
Distal part of medial toe	88.01 (10.37)	98.97 (10.29)	92.94 (11.56)	69.49 (21.24)	123.71 (6.99)	83.67 (21.32)
Middle part of middle toe	124.89 (10.85)	112.22 (3.2)	149.22 (6.87)	131.36 (5.85)	140.89 (12.49)	163.8 (22.6)
Distal part of middle toe	90.70 (8.39)	94.94 (7.26)	131.19 (6.45)	116.66 (12.07)	145.9 (6.89)	127.01 (8.43)
Proximal part of lateral toe	141.47 (11.52)	145.9 (14.33)	155.57 (11.87)	158.76 (9.5)	186.09 (11.91)	155.3 (20.14)
Distal part of lateral toe	102.75 (4.8)	126.07 (13.08)	125.4 (11.93)	103.92 (3.19)	156.14 (9.54)	118.84 (17.97)

Table 3.4 shows a regional analysis of the mean maximum pressures developed at various parts of the foot during a footstep, averaged for each bird over 5 runs. In general, the metatarsal pad is subject to lower pressures, with the highest pressures seen on the back and medial toe. This is illustrated graphically in Figure 3.3, a time - pressure curve of a typical footstep, showing the pressure values, and duration over which they were applied. High pressures are seen on the back toe, as the foot initially contacts the ground, dropping to zero as the footstep progresses. Low pressures are seen throughout on the metatarsal pad and proximal part of the middle toe. Higher pressures are seen initially on the proximal part of the lateral toe, but these decrease around midway through the footstep, as the pressure rises on the proximal part of the medial toe.

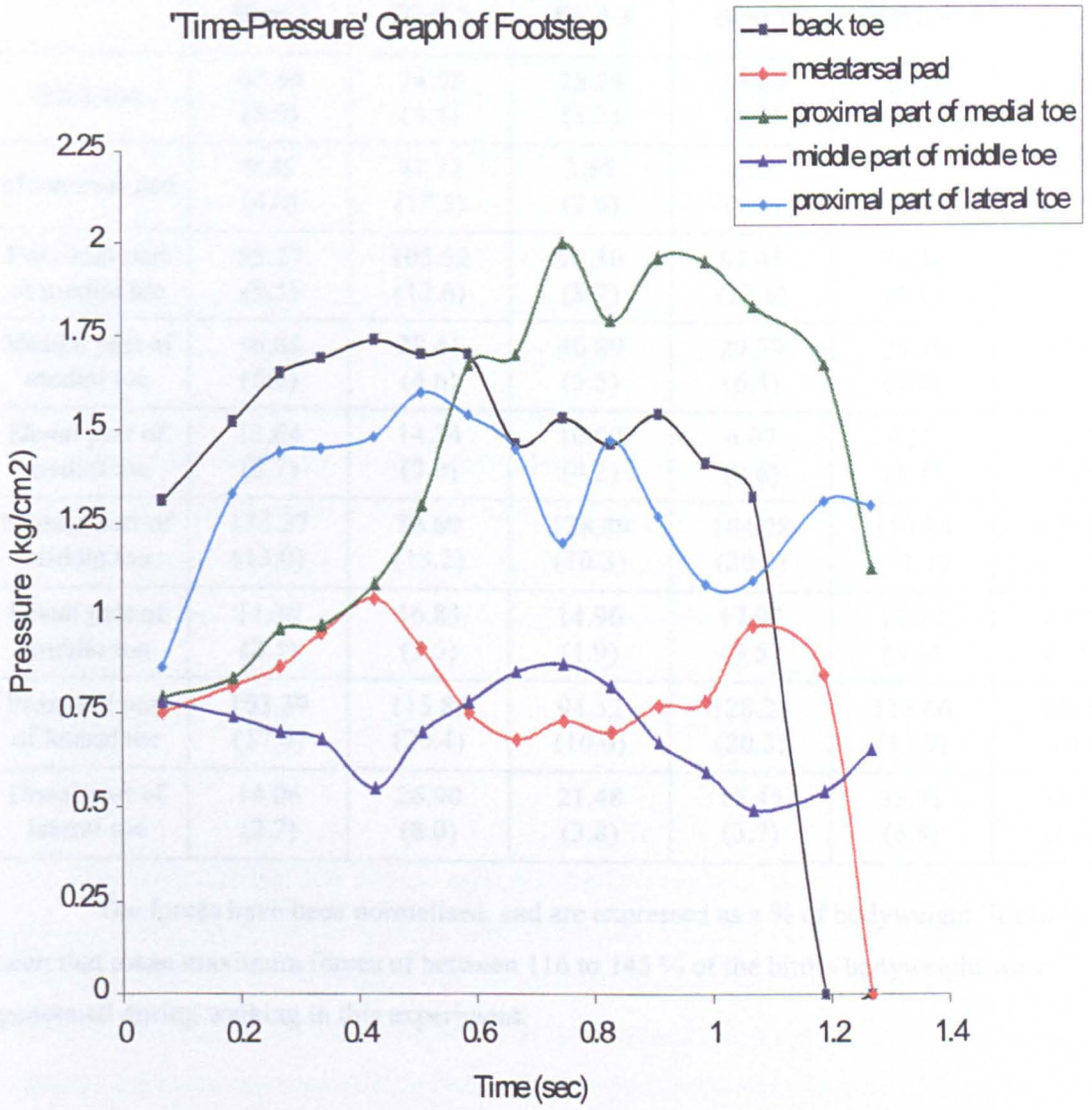


Figure 3.3: An example of a 'time-pressure' graph of a footstep, showing the initial sequence of loading. Pressure measurements arising from the distal parts of the toes later in the course of the footstep have been omitted to simplify the graph.

The areas over which the pressures were exerted were measured, and the formula force = pressure x area used to calculate the net forces being applied during normal walking. The maximum force at each point has been taken for each bird, meaned over 5 runs, and the results are summarised in Table 3.5.

Table 3.5: Regional analysis of mean maximum (net) vertical force, as a percentage of bodyweight (± SEM).

	Bird 1	Bird 2	Bird 3	Bird 4	Bird 5	Bird 6
Back toe	67.64 (8.0)	74.48 (4.4)	28.59 (3.2)	56.00 (6.8)	26.48 (1.1)	46.90 (4.2)
Metatarsal pad	9.45 (4.0)	91.72 (17.3)	3.85 (2.6)	1.24 (1.2)	23.03 (8.7)	38.22 (16.1)
Proximal part of medial toe	55.27 (9.5)	105.52 (12.6)	92.30 (8.7)	67.45 (13.8)	74.34 (6.1)	126.34 (15.8)
Middle part of medial toe	16.85 (5.6)	28.41 (4.6)	40.89 (5.5)	29.79 (6.4)	25.79 (2.8)	34.34 (8.5)
Distal part of medial toe	11.64 (2.7)	14.34 (3.0)	10.07 (4.2)	6.07 (2.6)	9.52 (1.4)	7.23 (3.2)
Middle part of middle toe	135.27 (13.0)	88.69 (13.2)	128.89 (10.3)	104.28 (20.3)	110.34 (11.1)	145.24 (35.4)
Distal part of middle toe	11.39 (2.1)	16.83 (5.5)	14.96 (1.9)	17.93 (3.5)	17.24 (3.3)	16.97 (1.6)
Proximal part of lateral toe	103.39 (17.0)	115.86 (20.4)	94.52 (10.0)	128.28 (20.3)	117.66 (13.9)	95.03 (20.1)
Distal part of lateral toe	14.06 (2.7)	26.90 (8.0)	21.48 (3.8)	15.45 (3.7)	35.31 (6.8)	16.41 (6.6)

The forces have been normalised, and are expressed as a % of bodyweight. It can be seen that mean maximum forces of between 116 to 145 % of the bird's bodyweight were generated during walking in this experiment.

3. 4 DISCUSSION

To be able to describe a normal walking pattern with any confidence, it is important to establish how variable the parameters are not only between birds, but also within a bird. The statistical significance of the bird component of variance in some of the measurements indicates that birds differ, however the variance within birds is also high, so that on average there would still be considerable overlap between large samples of observations from birds chosen at random. Should the variability between birds have been high, and that within birds low, then it would be difficult to confidently describe 'normal' ranges for the parameters under study. It will be interesting to compare the levels of variability in gait parameters shown by these normal healthy birds with the variability shown by broilers and

lame birds. It might be expected that the gait patterns in broilers and lame birds could be limited by structural or pathological changes in the musculoskeletal system, and therefore show lower variability, both between, and within birds.

As a consequence of the high variation between runs within a bird, the number of runs required to estimate a particular bird mean with even modest precision is large. The 95% ranges can be used to establish a range of values for the spatial parameters in the birds, however, and it can be seen that increasing the number of runs per bird does not greatly reduce the range. It is also possible that the variance within the birds due to fatigue, habituation or frustration, for example, might be greater than that estimated if the number of runs was increased over that used in the present study. In this study, the C'sV for measurements made on individual steps is not smaller than the C'sV between runs within birds, suggesting that using measurements from more than one step per run would improve the precision of a bird mean to the same extent as using steps from different runs. This could be facilitated by increasing the length of the recording plate, thereby increasing the minimum number of steps per run. An alternative would be to repeat runs over several days, however Holmes *et al* (1991), suggest that the benefits of doing extra trials on a single day outweigh the improvement in the estimate of error achieved by obtaining trials on different days.

When collecting data for gait analysis, it is important to try to control the subject's speed, as speed can affect some of the other gait parameters. This is difficult with domestic fowl, as any interference with the birds can alter their gait dramatically (Gentle and Corr 1995). As the birds were allowed to move freely, a wide range of speeds were seen; it was therefore necessary to determine the influence of speed on the other gait parameters measured in this study. This was done using regression analysis, and the observations were consistent with there being no difference between the 'between bird' and 'within bird' regressions. Although the results showed that step width and step angle were not significantly influenced by speed, this was not the case with cadence and step length. This is to be expected, as speed is a function of distance over time, distance being dependent on step length and number of steps. The regression coefficient produced for log cadence on log speed was greater than that produced for log step length on log speed (the slope for the line for cadence was steeper than that for length i.e. a greater rate of change for cadence than length, with increasing speed). In this sense, the birds appeared to increase speed by increasing step frequency more than by lengthening their steps, over the range of speeds in this study. This method of increasing speed mainly by increasing step frequency has also

been found in other small bipeds such as quail and guinea fowl, whereas larger bipeds such as ostriches show more of an increase in stride length (Gatesy and Biewener, 1991). In contrast, hopping bipeds such as crows increase speed by lengthening their stride, while keeping stride frequency fairly constant (Hayes and Alexander, 1983). In quadrupeds (mouse, rat, dog and horse), stride frequency increased linearly with speed during the walk and trot, but as the animal changed to a gallop, stride frequency remained nearly constant, further increases in speed resulting from increased stride length (Heglund *et al*, 1974).

The mean step length of 0.11m in this study suggests shorter steps than the average stride lengths of 0.28 m found previously by Farage-Elwar (1989) in 40 - day - old broilers, however the step widths (approximately 0.03m) were similar. In turkeys, Abourachid (1991) recorded stride lengths of 0.36m (+/- 0.03) in broad-breasted birds, and 0.3m (+/- 0.02) in traditional birds, as would be expected from the greater leg length of these larger birds. In studies where lameness was experimentally created in broilers (Farage-Elwar 1989), White Leghorn chicks (Sheets *et al* 1987), and rats (Clarke and Parker 1986), stride length was found to decrease together with a large increase in step width, suggesting that step width in particular is a useful measure of difficulty in walking. Sheets *et al* (1987) also showed that the foot was placed with the 3rd (medial) toe pointed inwards ('toe-in'), in normal chicks, as confirmed in this study, but in the lame birds, the feet pointed more in line with the direction of walking.

Of particular interest are the results of the plantar pressure measurements, as this aspect of gait has not been studied extensively in birds. The 'time-pressure' curve provides a visual display of changes in the loading pattern as the footstep progresses, similar to the pressure plots used in human clinical gait analysis (Franks *et al* 1983): it not only gives information on the magnitude of the pressures, but also on the duration of their application. Duration of application of pressure is very important, as high pressure sustained for a prolonged period, for example, is more likely to cause damage than a transient peak (Duckworth *et al* 1982). Such transient high pressures are seen on the back toe, as the foot initially contacts the recording surface. The highest plantar pressures during walking appear to be concentrated on the medial toes, and this is consistent with the birds maintaining their centre of gravity between the two legs. The metatarsal pad is subjected to the lowest pressures in general, as the total force is distributed over a greater area in this large, soft pad. Studies in the field of human gait analysis suggest that it is likely that each region of the foot has a different threshold pressure above which damage can occur, and so it is

important to attempt to establish 'danger' thresholds, and identify areas where damage may be more likely to occur (Betts *et al* 1980a, Cavanagh and Ulbrecht 1994). By identifying thresholds for different regions of the foot, it might be possible to predict whether lesions at particular sites are likely to be painful and therefore result in more severe lameness (Betts *et al*, 1980a). A good example of this is the finding that the metatarsal pad, an area commonly affected by lesions in birds, was subjected to lower pressures during most of the footstep. If this area has a low threshold for damage however, lesions could develop if abnormally high pressures are created in this area when birds are forced to stand in mesh floored cages, or when litter sticks to the pad in pens. It would therefore be useful to establish the normal pressure ranges for different regions of the foot in birds, and try to identify 'danger' thresholds above which the areas may be prone to damage.

Calculation of forces based on the pressure results obtained in this study in the chicken suggest that there is a similar relationship between peak vertical forces during walking and bodyweight to that seen in the human, where various parts of the footstep produce loads of the order of 120 - 150% of bodyweight (Betts *et al* 1980a, Whittle 1991). The loading is normally evenly distributed between each limb, as would be expected in bipeds (Gatesy and Biewener, 1991). These similarities between human and avian bipeds are to be expected based on the 'dynamic similarity hypothesis' proposed by Alexander and Jayes (1983), which suggests that animals will move in a dynamically similar fashion where possible, the aim being to minimise the energy requirement of movement (or 'cost of transport'). In contrast, studies on quadrupeds have shown lower peak forces, due to the different loading of the limbs. Bennett *et al* (1996) found peak vertical forces in the walking dog of the order of approximately 74 % of bodyweight, and Clarke (1995) describes peak forces in the walking rat of between 47.3 - 49.5 % of bodyweight, for the fore and hindpaw respectively. It should be noted, however, that comparing results from studies using different systems is not ideal because of the lack of absolute calibration between devices (Cavanagh and Ulbrecht, 1994).

The results demonstrate that the pedobarograph enables objective, quantitative information to be obtained on a variety of gait parameters in birds. The main advantage of the pedobarograph over many other gait analysis systems is the ability to measure plantar pressures, allowing comparisons to be made of pressure patterns and peak pressures between birds, between feet in individual birds, and even between different areas of the foot.

CHAPTER 4

A STUDY OF BROILERS: MORPHOLOGY AND GAIT ANALYSIS

4. 1 INTRODUCTION

As discussed in the General Introduction, the abnormal gait seen in broilers could be the result of pain, or of biomechanical problems associated with different types of conformation (or scaling), or both. This second experiment was designed to compare the gait patterns and morphology of different groups of broilers during growth, and again at the same final bodyweight.

The modern broiler has been selected for very rapid growth rates, to high end body weights. It has also been selected to produce more breast (i.e. pectoral) muscle, resulting in a change in conformation, since the breast increases in volume and becomes broader. Both of these changes can affect locomotion: the rapidly increasing bodyweight will put greater demands on the immature skeleton, and the change in shape could alter the magnitude and/or direction of the forces produced during walking.

This study had two main parts. Firstly, the pedobarograph was used to measure gait parameters in the broilers, to enable comparisons to be made between the gait patterns of the different types of bird. The gait was analysed weekly, so that changes in gait parameters during growth could also be investigated. Secondly, morphometric measurements were made each week to describe body conformation as the birds grew. Measurements were also made *post-mortem*, the birds having been culled at a bodyweight of approximately 2.4kg. This data was analysed to determine how the body components scaled during growth. Finally, the bones and joints were examined for underlying pathology, with particular reference to the common conditions of tibiotarsal torsion and tibial dyschondroplasia.

4. 2 MATERIALS AND METHODS

The pedobarograph used in this experiment is described in the General Materials and Methods section (Chapter 2.1).

Birds

Forty four day-old chicks were obtained from Ross Breeders, 20 'relaxed' chicks, and 24 'selected' (Ross 308 strain) chicks (described in Chapter 2.4). One of the relaxed chicks turned out to be male, and so his data were excluded from the final analysis, to avoid introducing confounding effects due to sexual dimorphism. The experiment was designed to compare the 2 strains of broiler, grown at two different rates to the same final bodyweight (a commercial cull weight of 2.4kg). Four groups were therefore used:

Group A - selected broilers, fed *ad libitum* (GpA)

Group B - relaxed broilers, fed *ad libitum* (GpB)

Group C - selected broilers, restricted-fed (GpC).

Group D - relaxed broilers, restricted-fed (GpD).

Each chick was individually identified by a coloured leg band, and 10 birds of the appropriate strain were randomly allocated to each group, except for Gp A which had 14. Extra chicks were included in GpA to allow for the higher mortality rates seen in *ad libitum*-fed selected birds (as the demands of the rapid growth put them under considerable physiological stress). The birds were reared on the floor on wood shavings, initially in chick boxes (width=39 cm, length=72 cm and height=31 cm) until day 7, and thereafter in floor pens. Unfortunately, a single room was not available to house all the birds, and so they were reared in two separate rooms, with the conditions in the two rooms being kept as similar as possible. The *ad libitum*-fed groups were kept in separate pens in one room, with approximately 0.26m^2 floor space per selected bird, and 0.21m^2 per relaxed bird. The restricted-fed birds were kept in separate pens in the second room, with an average of 0.22m^2 per bird. Although there were differences in the area per bird, the stocking density was sufficiently low to enable all the birds to move around freely. Average temperature in the rooms was maintained between 20.5-23.5 °C, and average relative humidity between 48.8- 57.7%.

Lighting patterns are known to have an effect on activity levels in poultry (Hester, 1994), and so these were carefully controlled. Standard broiler lamps (150W ceramic bulb, Pearlco I.P.O.) were kept on all day up to day 16, by which time the chicks were scattering away from the lamps. The room lighting was provided by two 250 W bulbs in each room; the 'daylength' was reduced from 23 hours on day 1 to 14 hours by day 5, thereafter being maintained on the 14 hours light: 10 hours dark regime.

Of the original 44 birds, one Gp A bird was culled at a few days old, when it began to show respiratory problems and general weakness, however no abnormality was found on *post-mortem* examination. The rest of the birds survived however, resulting in a slightly unbalanced data set.

Feeding regime

The birds were fed on the Roslin Broiler Starter diet throughout life (Appendix 4.1), with water available *ad libitum*. All chicks were fed *ad libitum* until day 11, after which Groups C and D were restricted-fed. As broilers are not usually restricted-fed, the Ross Parent Stock Male (PSM) protocol was applied (Appendix 2.2). Pair feeding was used, so that the same % restriction could be applied to both strains. (The PSM ration was compared to the *ad libitum* intake of the selected birds, and the % restriction calculated. The *ad libitum*

intake of the relaxed birds was measured, and the same % restriction then applied to the relaxed restricted group). The *ad libitum*-fed birds were given known amounts of food at 4 pm each day, and the quantity that remained uneaten after 24 hours was measured. The restricted-fed birds were fed two meals daily, the first meal of approximately half the estimated daily ration being fed at 9 am, the second at 4pm. Prior to the afternoon feed of the GpD birds, the food intake of the *ad libitum*-fed selected birds over the previous 24 hours was calculated, and compared to the recommended restriction ration. That day's % restriction could then be calculated, and the 'outstanding' amount fed to the GpD birds in their afternoon feed. Thus both the GpC and GpD birds were on the same percentage restriction as each other, based on the *ad libitum* levels of their 'strain-mates' (although half a day behind). The restricted-fed birds were observed during feeding times to ensure that there was no fighting, and that each had equal access to the food. Any birds showing markedly lower bodyweight gains than others in the group were separated at feeding time and given their ration individually.

On reaching approximately 2.4kg (at 6 weeks), the *ad libitum*-fed selected birds were culled, and restricted-fed selected birds continued on the PSM regime. The restricted-fed relaxed birds were then kept on 45% of *ad libitum*-fed relaxed birds intake. When the *ad libitum*–fed relaxed birds were culled, the restriction level for the remaining birds was estimated so as to maintain a similar weekly growth rate.

Figure 4.1 shows a bird from each group at 6 weeks of age.

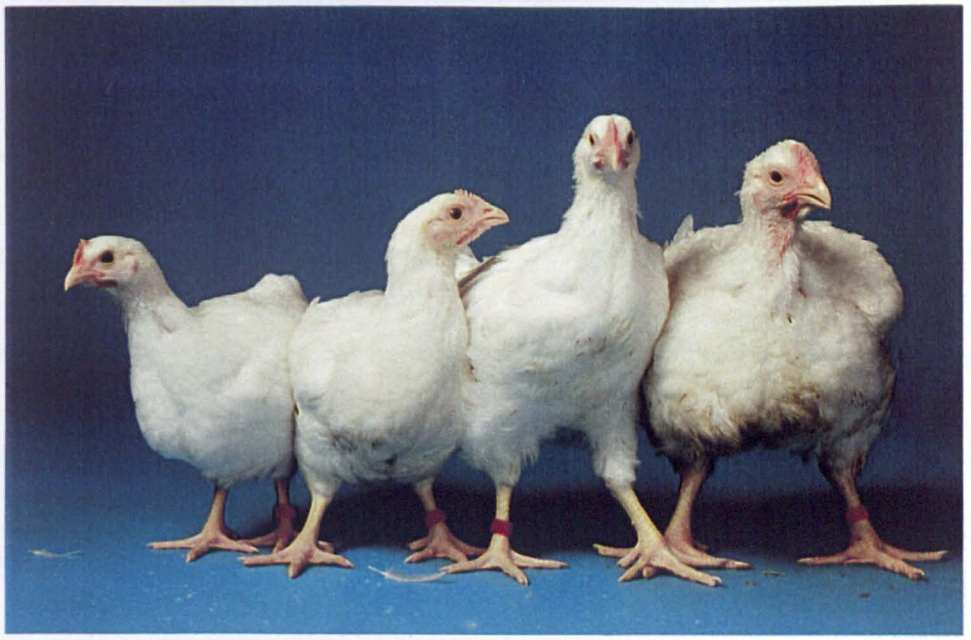


Figure 4.1: Photograph showing a bird from each of the 4 groups at 6 weeks of age (from left to right: Gp D, Gp C, Gp B and Gp A).

Testing Protocol

From a week old, the birds were familiarised with the runway. The chicks tended to stay together, and so it was easy to motivate them to walk along the runway, by placing one at a time at the opposite end to their companions. The motivation to walk has been discussed in Chapter 2.4, and it was with particular reference to this experiment that feed rewards were avoided. While the *ad libitum*-fed birds were usually satiated, the restricted-fed birds were always hungry and looking for food. Using feed rewards as an incentive to walk would have created very different levels of motivation between the groups. Attempts were made to equalise this motivation by feeding the restricted-fed birds their ration just before testing, so that they were more settled. This necessitated testing the birds in batches from the same group, rather than in a truly random manner, however both the order of the groups and of the birds within the groups was randomised.

Once a week, all the birds were weighed, and had the following morphometric measurements made. The following day, they were gait analysed on the pedobarograph (the gait parameters are described in Chapter 2.5).

Morphometric measurements: (illustrated in Figure 4.2)

Bodyweight – the birds were weighed (after the GpC and GpD birds were fed), using a Sartorius balance (BP34000, Sartorius AG, Germany), accurate to 0.1 g.

Girth - a piece of string was passed around the circumference of the thorax of the bird, tucked under the wings just behind the axillae, and it's length measured (accurate to 0.1mm)

Thigh muscle diameter - a piece of string was passed around the circumference of the top of the thigh, and its length measured.

Femur length – was measured from the point of the hip to the lateral condyle of the distal femur.

Tibiotarsal length – was measured from the lateral aspect of the tibial plateau to the lateral condyle of the tibiotarsus.

Tarsometatarsal length- was measured from the point of the hock to the large metatarsal pad.

Tarsometatarsal width – was measured mid-shaft, in both the lateral (lat), and craniocaudal (CrCd) direction.

The lengths of the bones of the hind limb were measured using calipers (RS Baty, R.S. Components, accurate to 0.1mm). Each length measurement was made twice, and the average used (estimated to be accurate to within 2mm).

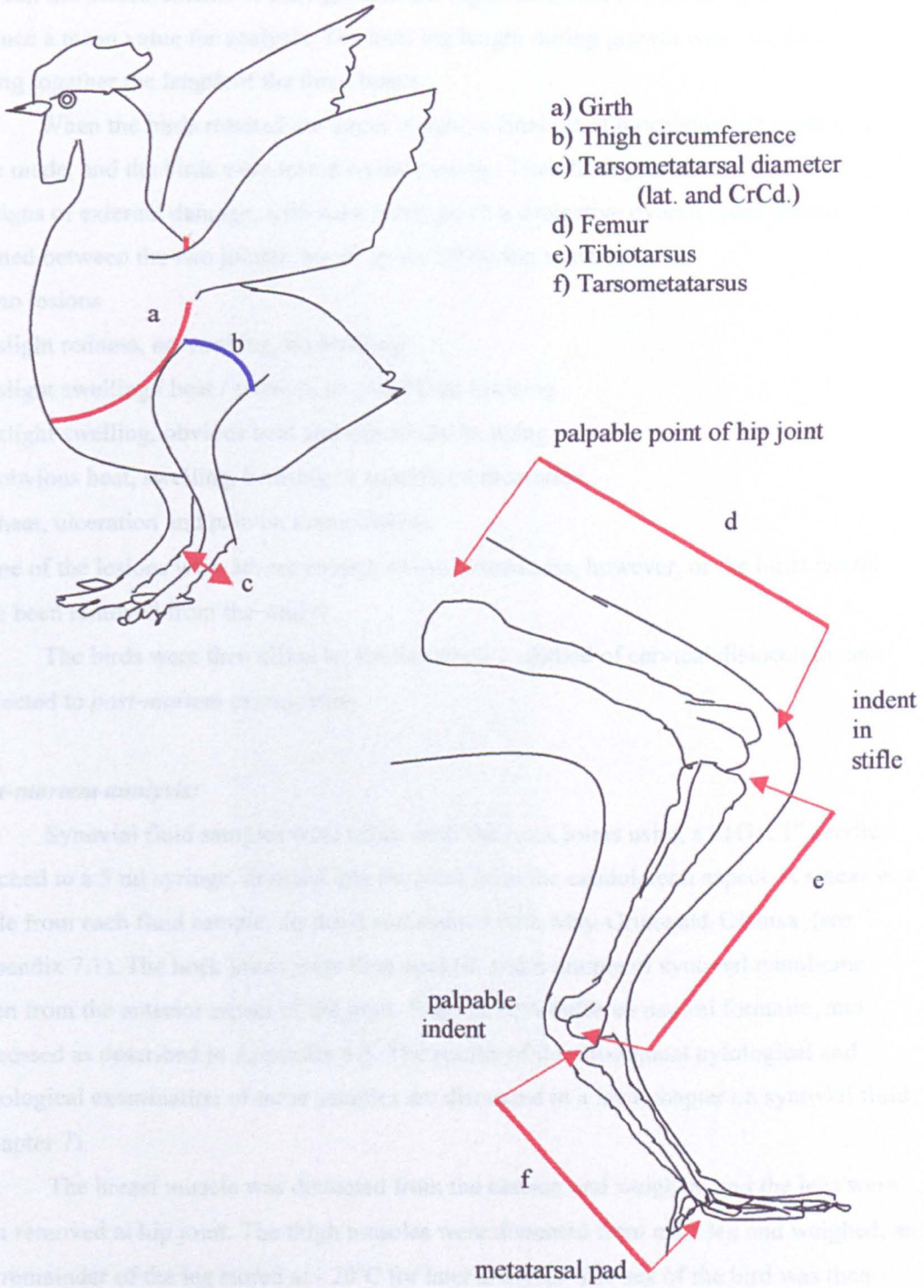


Figure 4.2: Morphometric measurements

All the measurements were made by the same person to reduce variability, as recommended by Burbridge and Pfeiffer (1998). No significant differences were found between the measurements of the right and left legs, and so the two values were averaged to produce a mean value for analysis. The total leg length during growth was calculated by adding together the length of the three bones.

When the birds reached the target weight, a final set of morphometric measurements were made, and the birds were tested on the runway. Their hock joints were then examined for signs of external damage, with each being given a subjective overall score (subsequently meaned between the two joints), based on the following scale:

0 = no lesions

1 = slight redness, no swelling, no bruising

2 = slight swelling / heat / redness, or superficial bruising

3 = slight swelling, obvious heat and superficial bruising

4 = obvious heat, swelling, bruising or superficial ulceration

5 = heat, ulceration and pain on manipulation.

(None of the lesions were severe enough to cause lameness, however, or the birds would have been removed from the study).

The birds were then killed by the Schedule 1 method of cervical dislocation, and subjected to *post-mortem* examination.

Post-mortem analysis:

Synovial fluid samples were taken from the hock joints using a 21G x 1" needle, attached to a 5 ml syringe, inserted into the joint from the caudolateral aspect. A smear was made from each fluid sample, air dried and stained with May-Grunwald-Giemsa (see Appendix 7.1). The hock joints were then opened, and a sample of synovial membrane taken from the anterior aspect of the joint, fixed in 10% buffered neutral formalin, and processed as described in Appendix 4.3. The results of the subsequent cytological and histological examination of these samples are discussed in a later chapter on synovial fluid (Chapter 7).

The breast muscle was dissected from the carcass and weighed, and the legs were then removed at hip joint. The thigh muscles were dissected from each leg and weighed, and the remainder of the leg stored at - 20°C for later analysis. The sex of the bird was then verified prior to disposal of the carcass. At a subsequent stage, the leg bones were defrosted overnight, the remaining tissues stripped off and discarded, and the bones weighed individually. Each bone was then radiographed whole in two views (lateral and craniocaudal), and the tibial plateau angles were measured from the radiographs, as illustrated in Figure 4.3. Images of the bones were then captured onto computer using the

NIH Image software on the PowerMac, as illustrated in Figure 4.4, which enabled the tibiotarsal torsions to be measured.

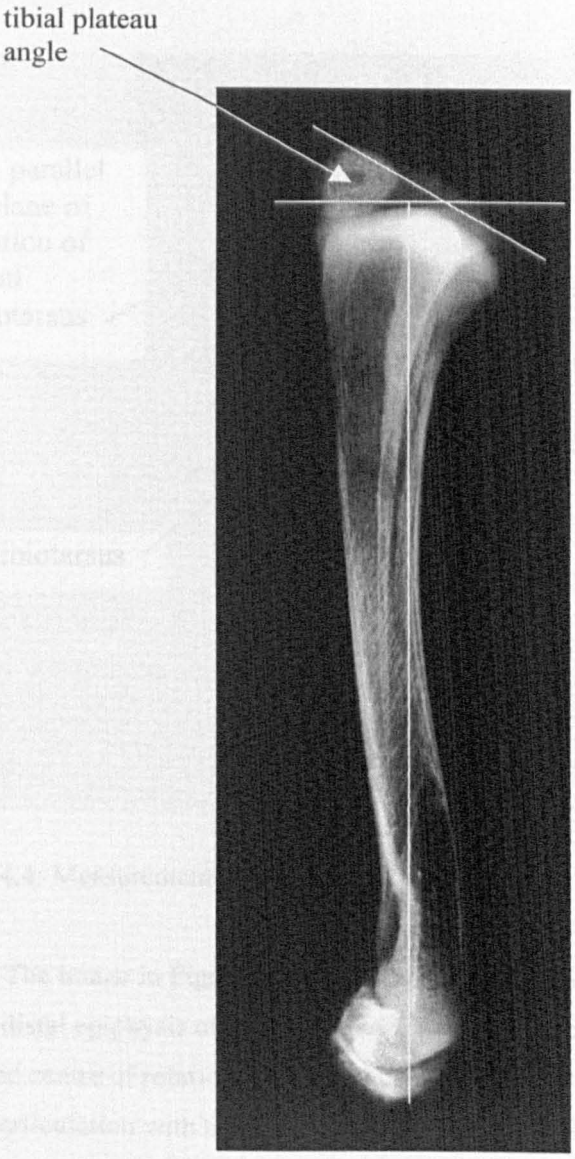


Figure 4.3: Measurement of the tibial plateau angle.

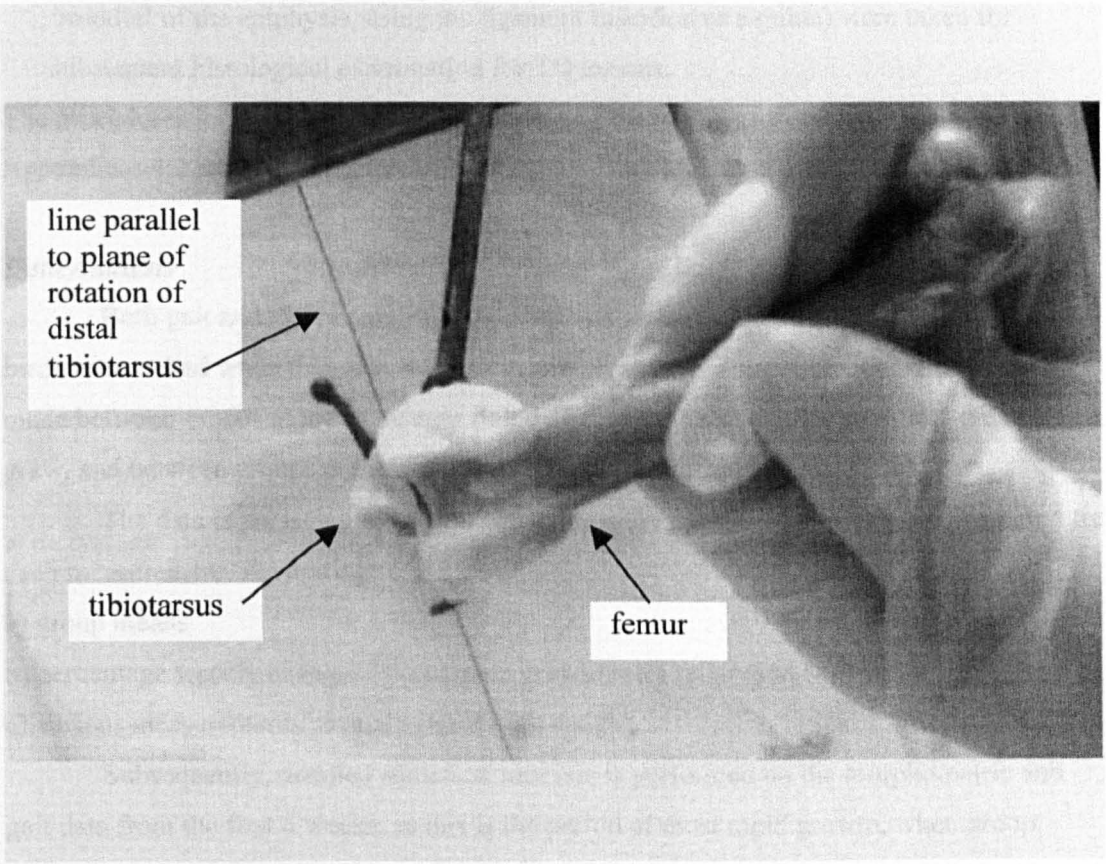


Figure 4.4: Measurement of tibiotarsal rotation.

The image in Figure 4.4 was set up as follows: a line was drawn on a piece of paper, and the distal epiphysis of the tibiotarsus lined up with this, along a plane parallel to the estimated centre of rotation of the joint. The femur was then placed to approximate its normal articulation with the tibiotarsus, and the 'joint' flexed back and forth, to establish the centre of rotation of the stifle joint. When the stifle was flexed approximately normally, the image was captured from above. Lines could then be drawn parallel to the estimated centre of rotation of each of the joints, and the angle between the two measured. In theory the lines should be parallel, and if this is not the case, the angle will give an indication of the degree and direction of torsion of the tibiotarsal bone. This method was developed by the present author, who found the method used by Duff and Thorp (1985a,b) to be difficult to apply, and it has since been adopted by other workers in the field (Wilson, unpublished data). Rotation of the distal tibiotarsus medially with respect to the proximal tibiotarsus is described as 'internal' rotation; where the distal tibiotarsus rotates laterally, this is described as 'external' rotation.

The tibiotarsi were then sectioned:

- mid-shaft cortical sections (1cm long) were sent for ashing, and
- proximal epiphyseal sections (2cm sections taken longitudinally down from the 'middle' of the epiphysis, using the ligament insertion as a guide) were taken for subsequent histological examination for TD lesions.

The procedures for bone ashing and preparation of the histological sections are described in Appendices 4.2 and 4.3 respectively.

Data Analysis

Both gait and morphometric measurements were made at weekly intervals, and the birds were culled when they reached approximately 2.4 kg. Thus comparisons could be made between groups at the same ages (but different weights), within groups as the birds grew, and between groups at the same final weight (but different ages).

The data is presented in several ways. Initially, a series of 3 graphs are presented for each measurement, illustrating:

a) group means

b) percentage weekly changes (to compare growth rates relative to bodyweight)

c) various measurements plotted against bodyweight

Subsequently, detailed statistical analysis is performed on the morphometric and gait data from the first 6 weeks, as this is the period of most rapid growth, when group differences are likely to be greatest. It also covers the commercial life span of the modern broiler. The cull data (when all groups had reached the same bodyweight) was also analysed in detail. As the groups were unbalanced, the group means and medians were generated using REML. Speed, cadence, step length, bodyweight, girth and leg length were all transformed to logarithms for analysis: the medians are presented, as a more resistant representation of the average, along with the approximate standard error of the median (#SE median) (Kendall and Stuart, 1963). The other parameters appeared to have a more normal distribution, and so the means (and standard errors) are given. Statistical significance between the groups was calculated by Students *t*-tests, using the maximum standard error of the differences between groups to give a conservative estimate.

Allometric analysis was carried out using bivariate linear regression analysis to investigate differences in scaling between the groups. The logarithm (log) of each variable of interest was plotted against the log of bodyweight, and the resulting regression coefficients compared (Swartz and Biewener, 1992). The growth data from the first 5 weeks, and cull weight, were analysed in this way, using REML, to take into account the hierarchical (birds / times / runs) and unbalanced nature of the data.

4. 3 RESULTS

To avoid excessive repetition in describing the results, the abbreviations > (greater than) and < (less than) are used, where appropriate, to describe differences between the groups, and the associated significance levels will be stated. The abbreviation # SE median is also used, to represent the first order approximation of the SE of median, calculated from (median x SE of log median).

Figures 4.5 – 4.10 are presented on the following pages. The graphs illustrate the mean values of the variables for each group (with bars illustrating ± 1SD included in the 'a' graphs).

Figure 4.5 (a): Bodyweight

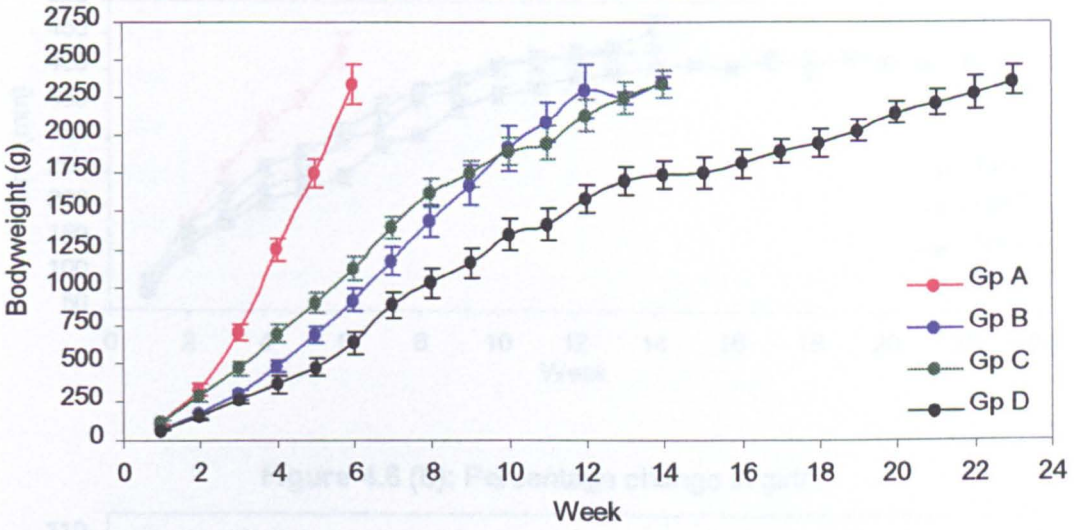


Figure 4.5 (b): Percentage change in bodyweight

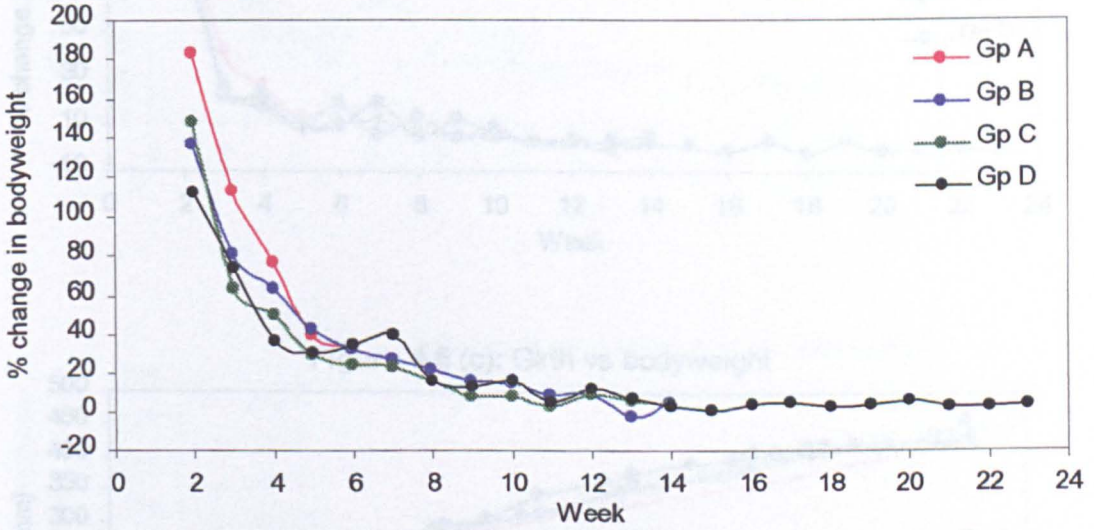


Figure 4.5 (a) shows the mean weekly bodyweights for each group (± SD). The Gp A birds had much steeper growth curves than the other 3 groups. The weekly increase in bodyweight appears to be similar between the Gp B and Gp C birds, while the increase in the Gp D birds was much less each week.

Figure 4.5 (b) shows the mean weekly percentage change in bodyweight. The greatest weekly percentage changes in bodyweight appear to have occurred in the first 5 weeks of growth, and thereafter the differences between the groups were small. The GpA birds showed the greatest % changes up to about 4 weeks, after which the relationship between the groups was more variable.

Figure 4.6 (a): Girth

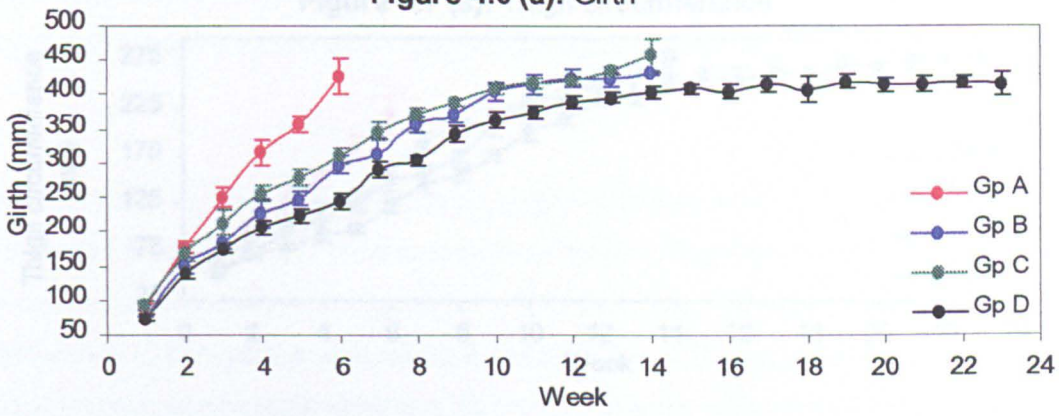


Figure 4.6 (b): Percentage change in girth

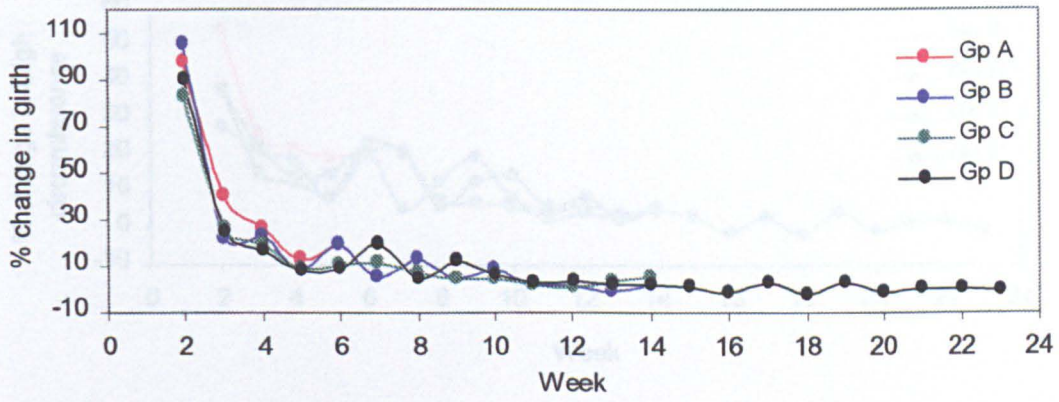


Figure 4.6 (c): Girth vs bodyweight

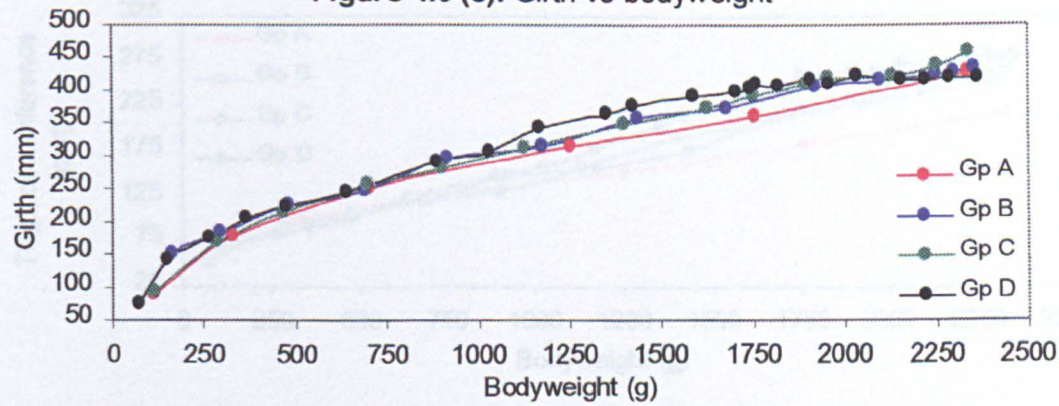


Figure 4.6 (a-c). Graph 4.6a (group means$\pm$ SD) shows that the patterns of girth development were very similar to those of bodyweight, as were the relationships between the groups at each stage. The largest differences between the groups in terms of % change in girth appeared to occur in the first 5 weeks (graph 4.6b). It is also obvious that girths of the groups were very similar at the same bodyweight (graph 4.6c).

Figure 4.7 (a): Thigh circumference

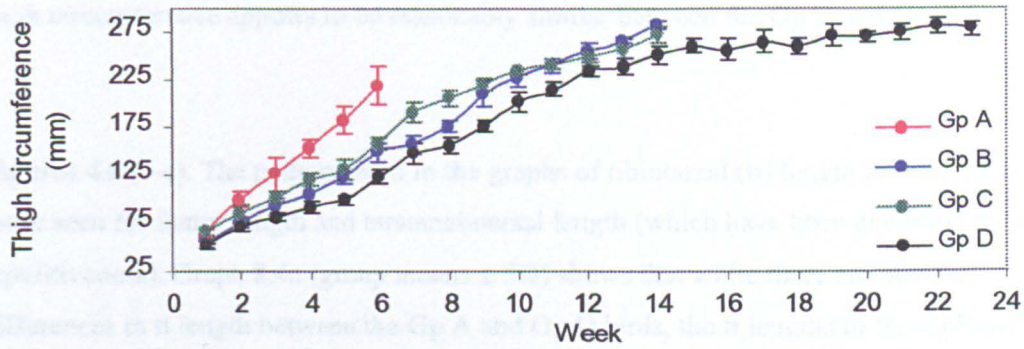


Figure 4.7 (b): Percentage change in thigh circumference

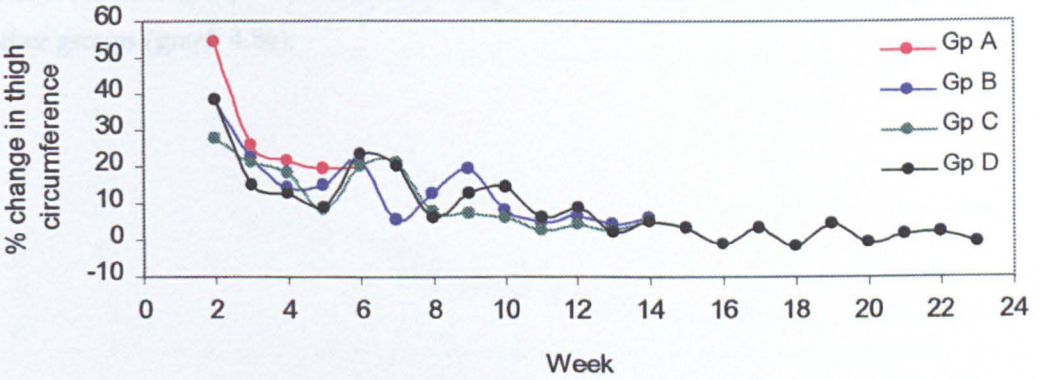
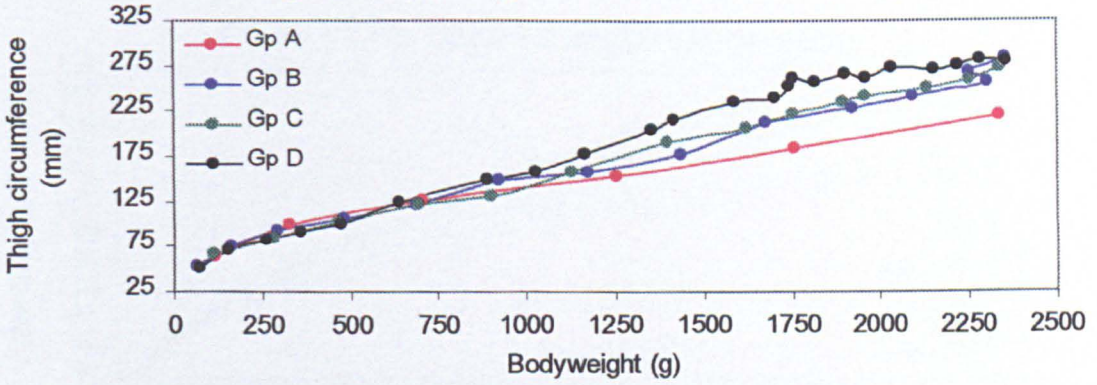


Figure 4.7 (c): Thigh circumference vs bodyweight



Figures 4.7 (a-c). Graph 4.7a shows the mean values for each group (± SD) - again, the trends for weekly thigh circumference were similar to those for weight and girth. The percentage change in weekly thigh circumference appeared to be more variable, with differences between the groups being apparent for the first 12 weeks (graph 4.7b). The graph of thigh circumference against bodyweight (4.7c) shows little difference between the groups when the birds weighed less than 1kg. Thereafter, the GpA birds had a smaller mean thigh circumference, and the Gp D birds a larger mean thigh circumference, in relation to

their bodyweight, compared to the other groups. The relationship between bodyweight and thigh circumference appears to be reasonably similar between the Gp B and Gp C birds.

Figures 4.8 (a-c). The patterns seen in the graphs of tibiotarsal (tt) length are similar to those seen for femur length and tarsometatarsal length (which have been excluded, to avoid repetitiveness). Graph 8.4a (group means ± SD) shows that while there are obvious differences in tt length between the Gp A and Gp D birds, the tt lengths of the GpB and GpC birds are very similar at the same age. The weekly changes within the groups were similar to those seen in the previous graphs, with the greatest % weekly changes most obvious in the first 6 weeks. The Gp A birds had markedly shorter tibiotarsi at the same bodyweight than other groups (graph 4.8c).

Figure 4.8 (a): Tibiotarsal Length

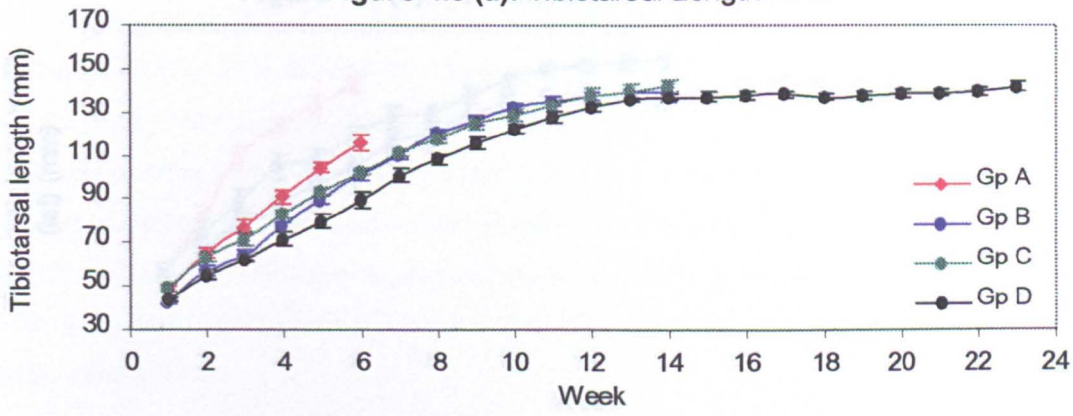


Figure 4.8 (b): Percentage change in tibiotarsal length

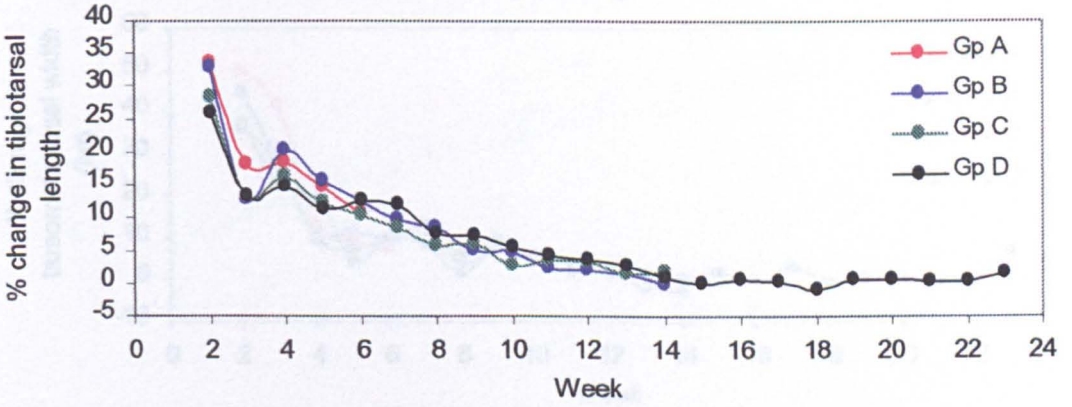


Figure 4.8 (c): Tibiotarsal length vs bodyweight

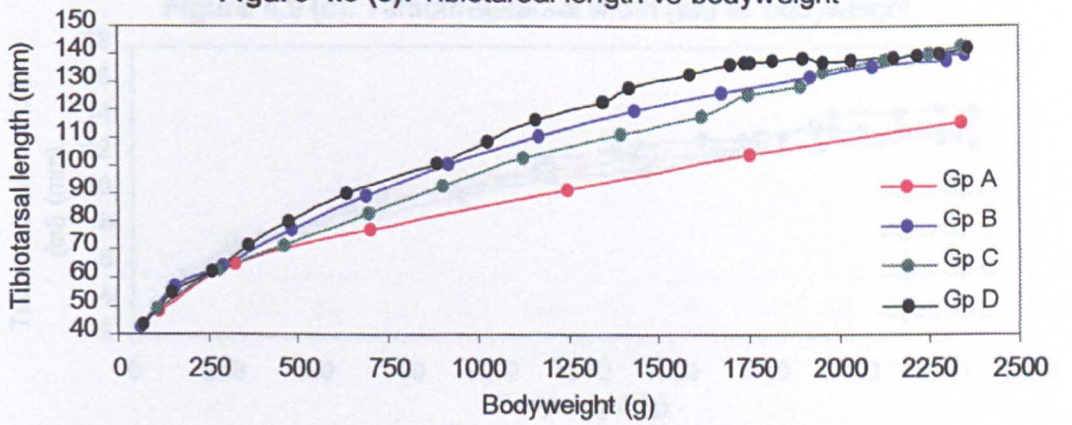


Figure 4.9 (a): Tarsometatarsal width (lateral)

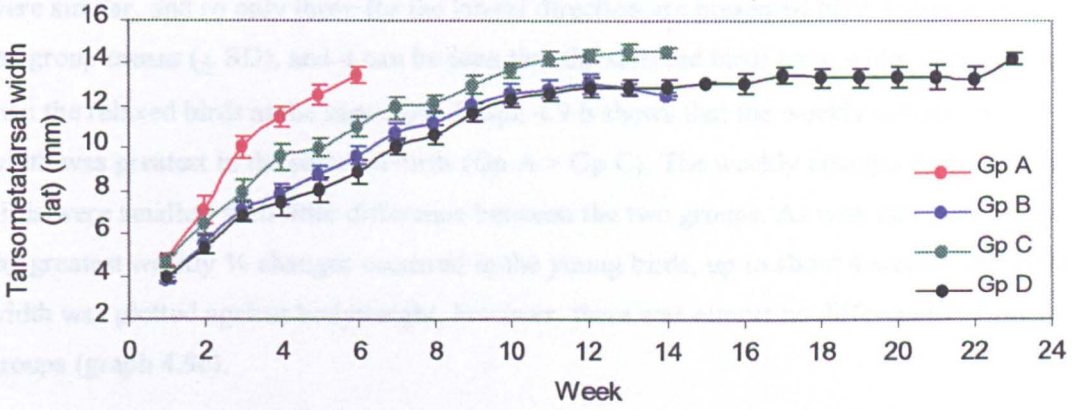


Figure 4.9 (b): Percentage change in tarsometatarsal width (lat)

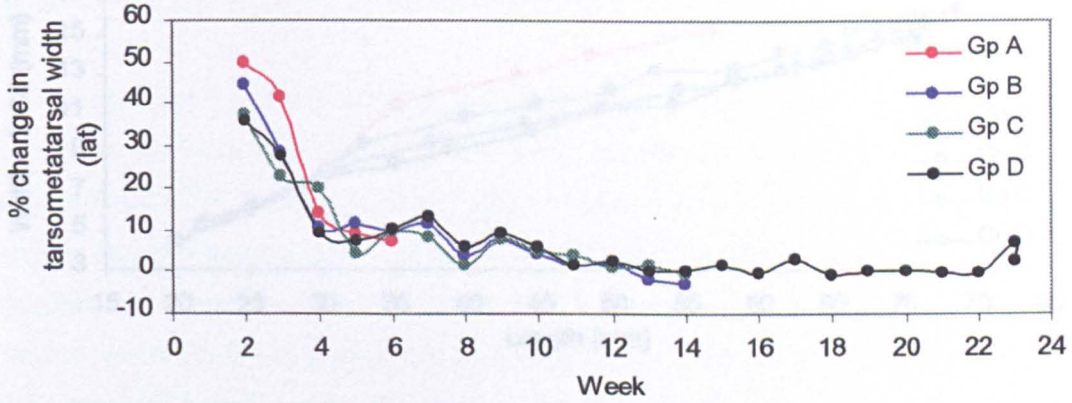


Figure 4.9 (c): Tarsometatarsal width (lat) vs bodyweight

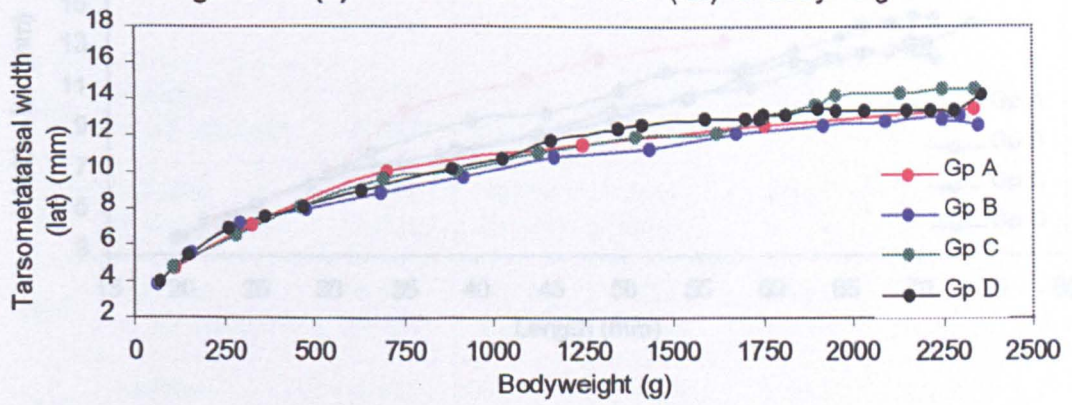


Figure 4.9 (a-c) Graphs of tarsometatarsal (tmt) width in a lateral and craniocaudal direction were similar, and so only those for the lateral direction are presented here. Graph 4.9a shows the group means (± SD), and it can be seen that the selected birds have wider tarsometatarsi than the relaxed birds at the same age. Graph 4.9 b shows that the weekly change in tmt width was greatest in the selected birds (Gp A > Gp C). The weekly changes in the relaxed birds were smaller, with little difference between the two groups. As with other parameters, the greatest weekly % changes occurred in the young birds, up to about 4 weeks. When tmt width was plotted against bodyweight, however, there was almost no difference between the groups (graph 4.9c).

Figure 4.10 (a): Tarsometatarsal length vs width (CrCd)

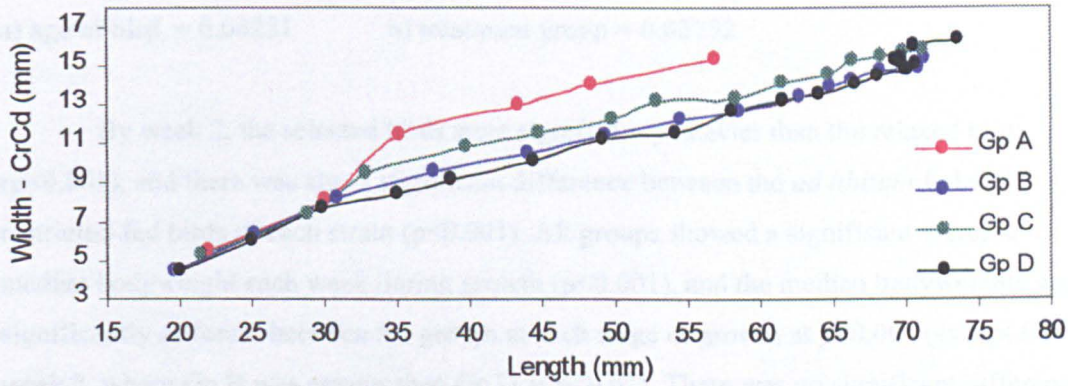
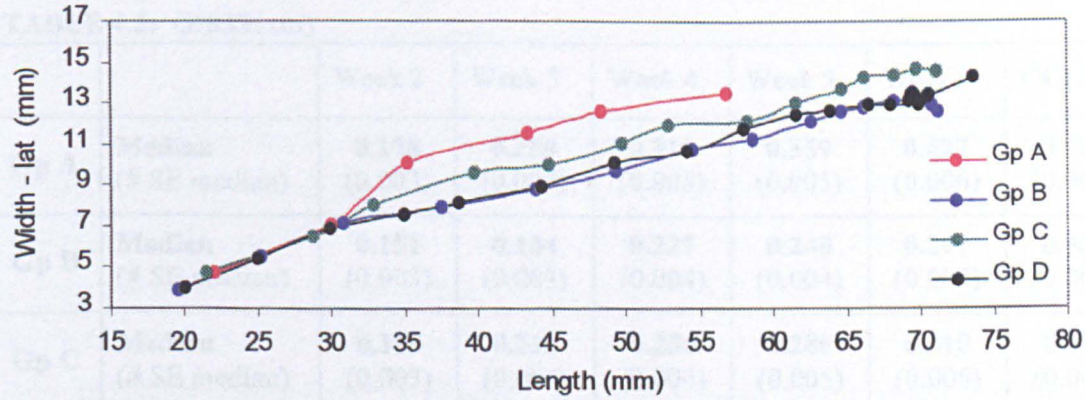


Figure 4.10 (b): Tarsometatarsal length vs width (lat)



Figures 4.10 (a-c). The Gp A birds had considerably greater tmt widths for a given length of bone than the birds in the other 3 groups. The tmt width to length ratio of the Gp C birds also appeared to be greater than those of the relaxed birds (with little difference in the ratio between the Gp B and Gp D birds).

TABLE 4.1: BODYWEIGHT (kg)

		Week 2	Week 3	Week 4	Week 5	Week 6	CULL
Gp A	**Median** (# SE median)	**0.329** (0.008)	**0.705** (0.018)	**1.252** (0.032)	**1.755** (0.045)	**2.339** (0.060)	**2.339** (0.060)
Gp B	**Median** (# SE median)	**0.163** (0.005)	**0.295** (0.009)	**0.484** (0.001)	**0.691** (0.020)	**0.919** (0.027)	**2.372** (0.069)
Gp C	**Median** (# SE median)	**0.282** (0.008)	**0.464** (0.014)	**0.694** (0.020)	**0.902** (0.026)	**1.126** (0.033)	**2.345** (0.069)
Gp D	**Median** (# SE median)	**0.151** (0.005)	**0.263** (0.008)	**0.358** (0.011)	**0.471** (0.014)	**0.634** (0.002)	**2.365** (0.073)

Maximum SE of difference of log medians for same level of factor:

a) age of bird = 0.04251 b) treatment group = 0.02752

By week 2, the selected birds were significantly heavier than the relaxed birds ($p<0.001$), and there was also a significant difference between the *ad libitum*-fed and restricted-fed birds of each strain ($p<0.001$). All groups showed a significant increase in median bodyweight each week during growth ($p<0.001$), and the median bodyweights were significantly different between the groups at each stage of growth at $p<0.001$ (except for week 3, where Gp B was greater then Gp D at $p<0.01$). There was no significant difference in bodyweight between any of the groups at the selected cull weight.

TABLE 4.2: GIRTH (m)

		Week 2	Week 3	Week 4	Week 5	Week 6	CULL
Gp A	**Median** (# SE median)	**0.178** (0.003)	**0.250** (0.004)	**0.315** (0.005)	**0.359** (0.005)	**0.427** (0.006)	**0.427** (0.006)
Gp B	**Median** (# SE median)	**0.151** (0.003)	**0.184** (0.003)	**0.227** (0.004)	**0.248** (0.004)	**0.297** (0.005)	**0.431** (0.007)
Gp C	**Median** (# SE median)	**0.169** (0.003)	**0.213** (0.004)	**0.256** (0.004)	**0.280** (0.005)	**0.310** (0.005)	**0.449** (0.008)
Gp D	**Median** (# SE median)	**0.141** (0.002)	**0.176** (0.003)	**0.206** (0.004)	**0.223** (0.004)	**0.243** (0.004)	**0.418** (0.007)

Maximum SE of difference of log medians for same level of factor:

a) age of bird = 0.02481 b) treatment group = 0.02275

All groups showed a significant increase in median girth each week during growth ($p<0.001$), with Gp A > Gp C > Gp B > Gp D. In the first 6 weeks of life, all groups had significantly different median girths from each other at $p<0.001$, with a few exceptions:

- at week 2, when Gp A > Gp C at $p<0.05$, and Gp B > Gp D at $p<0.01$,
- at week 3, when there was no significant difference between Gp B and Gp D, and
- at week 6, when there was no significant difference between Gp B and Gp C.

At cull, there was no significant difference in median girth between birds in Gp's A, B or D. Group C birds were not significantly different in median girth from Gp A or Gp B birds at cull weight, but had significantly larger median girths than Gp D birds ($P<0.001$).

TABLE 4.3: LEG LENGTH (m)

		Week 2	Week 3	Week 4	Week 5	Week 6	CULL
Gp A	**Median** (# SE median)	**0.104** (0.001)	**0.172** (0.001)	**0.206** (0.002)	**0.234** (0.002)	**0.266** (0.002)	**0.266** (0.002)
Gp B	**Median** (# SE median)	**0.093** (0.001)	**0.143** (0.001)	**0.173** (0.001)	**0.201** (0.002)	**0.224** (0.002)	**0.307** (0.003)
Gp C	**Median** (# SE median)	**0.104** (0.001)	**0.156** (0.001)	**0.186** (0.002)	**0.208** (0.002)	**0.230** (0.002)	**0.319** (0.003)
Gp D	**Median** (# SE median)	**0.094** (0.001)	**0.137** (0.001)	**0.159** (0.001)	**0.178** (0.002)	**0.202** (0.002)	**0.316** (0.003)

Maximum SE of difference of log medians for same level of factor:
a) age of bird = 0.01270 b) treatment group = 0.009741

Median leg length increased significantly in all groups each week ($p<0.001$) during growth. Gp A birds had significantly greater median leg length than all other groups up to 6 weeks ($p<0.001$), except for Gp C at 2 weeks, where there was no significant difference. Gp C birds had significantly greater median leg length than Gp B or Gp D birds at all stages in the first 6 weeks ($p<0.05$-0.001). Gp B birds had significantly greater median leg length than Gp D birds ($p<0.01$-0.001) at all stages in the first 6 weeks, apart from at 2 weeks.

At cull weight, Gp A birds had significantly shorter median leg length than all other groups ($p<0.001$). The Gp B birds had significantly shorter median leg length than Gp C birds ($p<0.01$) and the Gp D birds ($p<0.05$). There was no significant difference in median leg length of Gp C and Gp D birds at cull weight.

Figure 4.11: Speed

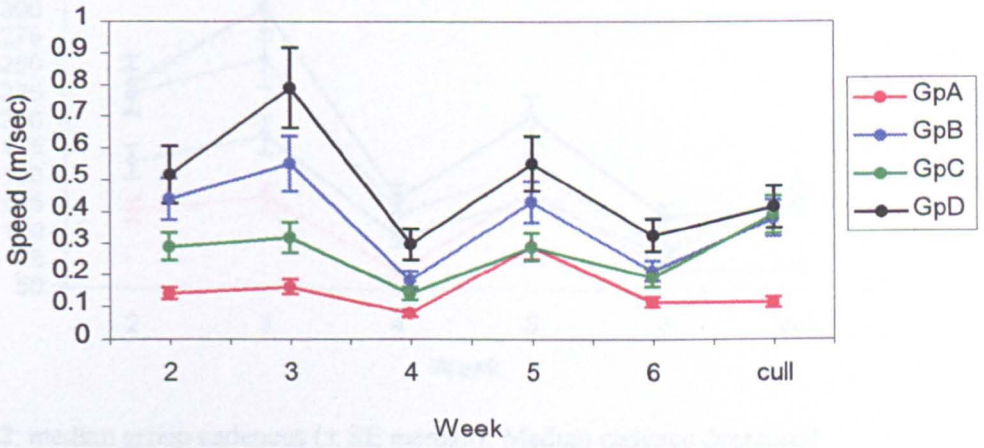


Figure 4.11: median group speeds (± SE median). At cull, Gp A birds showed a significantly lower median speed than all other groups ($p<0.001$), but there was no significant difference in median speed between the birds in Gp's B, C or D.Median speed was very variable, and there was no significant overall change as the birds grew. At most stages during growth median speed of GpD > GpB > GpC > GpA.

During growth, Gp A birds median speed was significantly lower than all the other groups ($p<0.05$-0.001) at all times except week 5, when it was significantly lower than only the Gp D birds ($p<0.01$). Gp C birds also had significantly slower median speeds than Gp D birds ($p<0.05$-0.001) up to 6 weeks, but not at cull. There were no significant differences in median speed between birds in Gp C and B (except at week 3, $p<0.05$). The relaxed groups showed no significant differences in median speed at any stage.

At cull weight, Gp A birds showed a significantly lower median speeds ($p<0.001$) than all other groups, but there was no significant difference in median speed between the birds in Gp's B, C or D.

Figure 4.12: Cadence

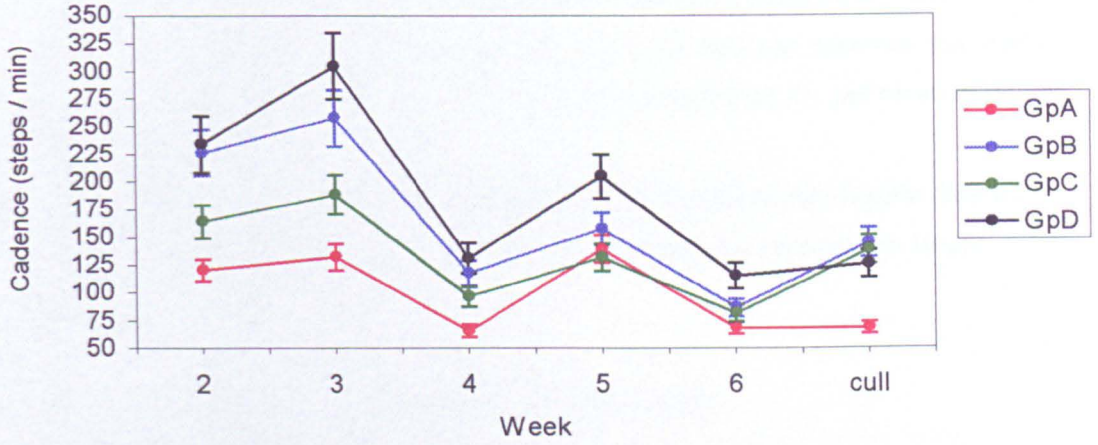


Figure 4.12: median group cadences (± SE median). Median cadence decreased significantly ($p < 0.01$-0.001) with age, in all groups except Gp C. At most stages during growth, GpD > GpB > GpC > GpA (as with speed). During growth, the median cadence of Gp A birds was significantly lower than Gp D birds throughout the first 6 weeks ($p<0.05$-0.001). It was also significantly lower than Gp B birds ($p<0.001$) and Gp C birds ($p<0.05$) for the first 4 weeks. Gp C birds were significantly lower than Gp B ($p<0.05$) at 2-3 weeks, and Gp D birds ($p<0.05$-0.01) from 2-6 weeks, but no different thereafter. There was no significant difference in median cadence at any stage between the relaxed groups.

Figure 4.13: Step Length

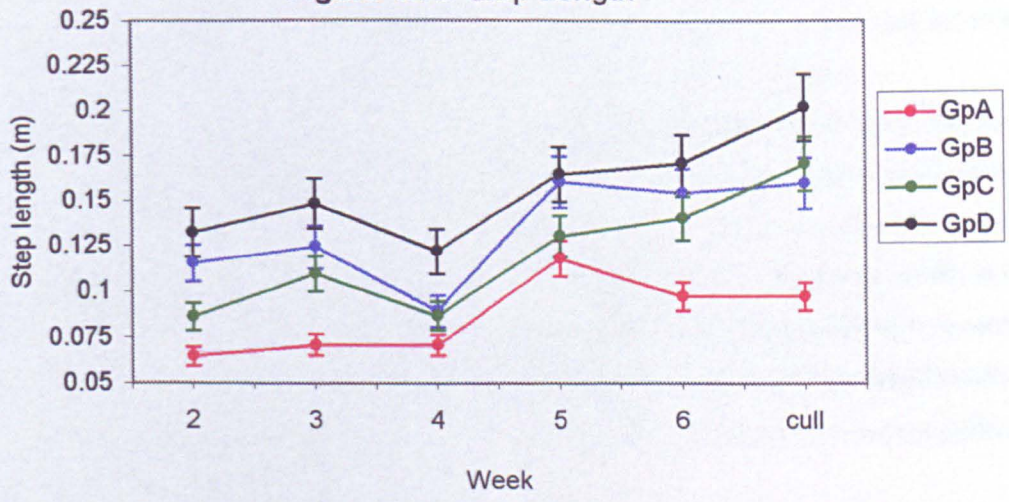


Figure 4.13: median group step lengths (± SE median). Median step length showed a significant increase as the birds grew (Gp B $p<0.05$, Gp's A and D, $p<0.01$, Gp C $p< 0.001$). At all stages during growth, median step length was greater in Gp D > Gp B > Gp C > Gp A.

Gp A birds took significantly shorter steps throughout growth than Gp D birds, and

Gp B birds, except at week 4 ($p<0.05$-0.001). Comparisons of median step length during growth between Gp A and C birds is more variable: Gp A birds always took shorter steps than Gp C birds, but not always significantly so. Gp C birds took significantly shorter steps than Gp D birds ($p<0.01$-0.05) for the first 4 weeks only. On only one occasion was median step length significantly different between the relaxed groups (week 4), and between the Gp C and Gp B birds (week 2).

At cull weight, Gp A birds had significantly shorter median step lengths than all other groups ($p<0.001$), but there was no significant difference in median step length between the birds in Gp's B, C or D.

Figure 4.14: Step Width

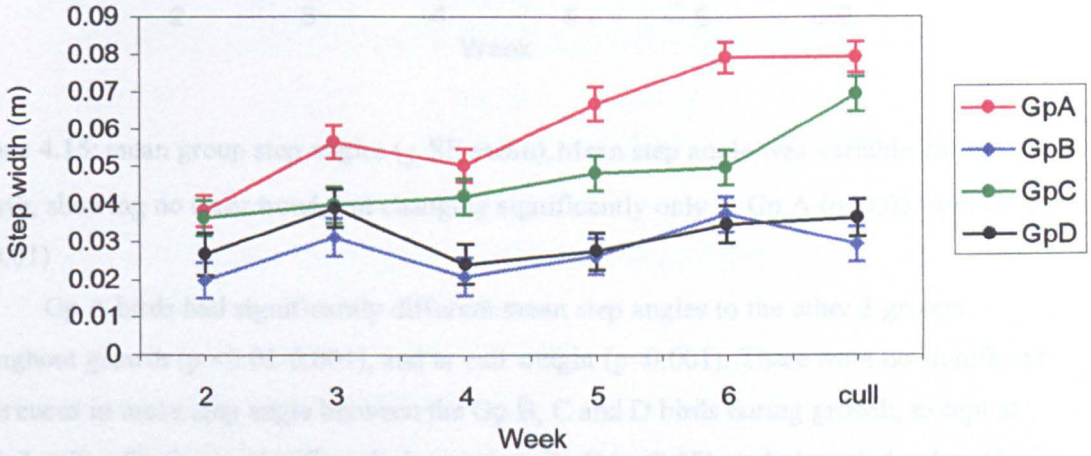


Figure 4.14: mean group step widths (±SE mean). Mean step width increased overall as the birds grew: this increase was significant in the selected groups ($p < 0.001$), but not in the relaxed groups. In general, at most stages, Gp A > Gp C > Gp D > Gp B.

During growth, mean step width was significantly greater in the Gp A than the Gp C birds at weeks 3 and 5 ($p<0.05$), and week 6 ($p<0.001$). Mean step width was significantly greater in Gp A birds than Gp B birds throughout ($p<0.05$ - 0.001), and than Gp D birds from 3 weeks onwards ($p<0.05$-0.001). The relationship between mean step width in Gp C and B birds was variable. The relaxed groups showed no significant difference in mean step width at any stage during growth. At cull weight, the selected groups had significantly higher ($p< 0.001$) mean step widths than the relaxed groups, but there was no significant difference within the strains.

Figure 4.15: Step Angle (degrees)

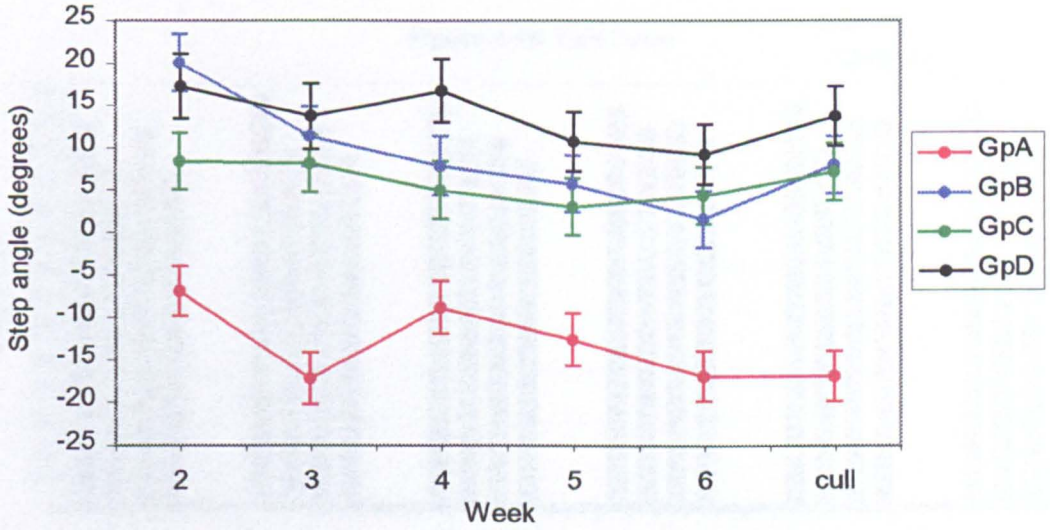


Figure 4.15: mean group step angles (± SE mean). Mean step angle was variable during growth, showing no clear trend, but changing significantly only in Gp A ($p<0.05$) and Gp B ($p<0.01$)

Gp A birds had significantly different mean step angles to the other 3 groups throughout growth ($p <0.01$-0.001), and at cull weight ($p<0.001$). There were no significant differences in mean step angle between the Gp B, C and D birds during growth, except at week 2, when Gp C was significantly lower than Gp B ($p<0.05$), and at week 4, when Gp C was significantly lower than Gp D ($p<0.05$). There were no significant differences in mean step angle between the Gp B, C and D birds at cull.

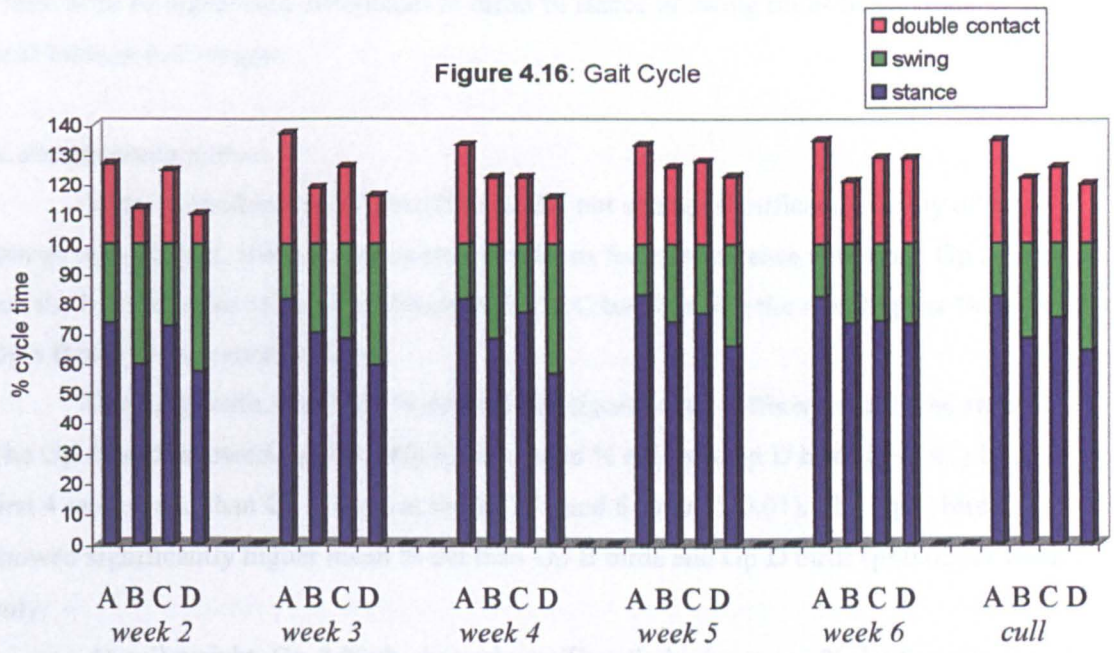


Figure 4.16: Illustrates the 'mean' gait cycle for each group of birds, defining the periods spent in swing, stance and double contact phases.

% Stance and Swing times

Mean % stance time did not change significantly during growth in any of the four groups, and therefore neither did mean % swing time. The overall trend in % stance time was to increase with age, and in general, Gp A was highest, then Gp C > Gp B > Gp D. The trend with % swing time was therefore to decrease with increasing age, and in general, Gp D > Gp B > Gp C > Gp A.

During growth, the selected groups showed no significant difference in mean % stance or mean % swing times, except at week 3 ($p<0.05$). Gp A birds had significantly greater mean % stance times, and smaller mean % swing times than Gp D birds ($p<0.01$-0.001) at all times except 6 weeks, and than Gp B birds up to week 4 ($p<0.05$).

Gp B birds had significantly lower mean % stance times than Gp C birds at week 2, and significantly higher mean % stance times Gp D birds at week 3 during growth ($p<0.05$). Gp B birds had a significantly higher mean % swing times than Gp C birds at week 2 only ($p<0.05$). Gp B had significantly lower mean % swing times than Gp D birds at weeks 3-4 only ($p <0.05$). Gp C birds had significantly lower mean % swing times than Gp D birds at weeks 2, 4 and 5 ($p<0.05$-0.001).

At cull weight, the selected groups showed no significant difference in mean % stance or mean % swing times. Gp A birds had a significantly greater mean % stance time

and a significantly shorter mean % swing time than Gp B ($p<0.05$) and Gp D ($p<0.01$) birds. There were no significant differences in mean % stance or swing times between the Gp B, C or D birds at cull weight.

% double contact time:

Mean % double contact time (% dct) did not change significantly in any of the groups with growth, although the overall trend was for it to increase with time. Gp A birds had the highest mean % dct at all times, with Gp C birds having the next highest % dct, then Gp's B and D were more variable.

During growth, the mean % dct was not significantly different within the strains. The Gp A birds showed significantly higher mean % dct than Gp D birds ($p<0.01$) for the first 4 weeks, and than Gp B birds at weeks 2, 3 and 6 ($p<0.05$-0.01). The Gp C birds showed significantly higher mean % dct than Gp B birds and Gp D birds ($p<0.05$) at week 2 only.

At cull weight, Gp A birds showed significantly higher mean % dct than Gp B and Gp D birds ($p<0.05$). There was no significant difference in mean % dct between the selected groups, nor between the Gp B, Gp C and Gp D birds at cull.

TABLE 4.4: Cull Data – breast muscle mass, thigh muscle mass, bone mass and hock scores.

		Gp A	Gp B	Gp C	Gp D	Max. SE of differences
Total breast muscle mass (kg)	**Median** (# SE median)	**0.443** (0.012)	**0.346** (0.011)	**0.479** (0.015)	**0.379** (0.012)	0.0462
% breast muscle mass	**Mean** (SE)	**19.02** (0.397)	**14.14** (0.453)	**20.13** (0.453)	**16.03** (0.478)	0.6760
Total thigh muscle mass (kg)	**Median** (# SE median)	**0.260** (0.005)	**0.226** (0.005)	**0.268** (0.006)	**0.244** (0.006)	0.0325
% thigh muscle mass	**Mean** (SE)	**11.14** (0.182)	**9.25** (0.207)	**11.25** (0.207)	**10.27** (0.219)	0.3090
Total leg bone mass (kg)	**Median** (# SE median)	**0.090** (0.001)	**0.075** (0.001)	**0.105** (0.002)	**0.083** (0.001)	0.0261
% leg bone mass	**Mean** (SE)	**3.847** (0.055)	**3.165** (0.062)	**4.541** (0.062)	**3.504** (0.065)	0.0925
Breast / Thigh muscle ratio	**Mean** (SE)	**1.708** (0.036)	**1.530** (0.041)	**1.800** (0.041)	**1.556** (0.043)	0.0608
Hock Score	**Mean** (SE)	**3.38** (0.239)	**1.07** (0.272)	**1.55** (0.272)	**0.89** (0.287)	0.4060

Breast muscle

There was no significant difference in breast muscle mass (total or %) between the selected groups. The selected birds had significantly higher breast muscle mass (total and %) than the relaxed birds ($p< 0.001$). There was no significant difference in total breast muscle mass between relaxed groups, but the Gp D birds had a significantly higher % breast muscle mass than the Gp B birds ($p<0.01$).

Thigh muscle

The selected groups had significantly greater thigh muscle mass (total and %) at cull than the relaxed groups ($p<0.05$-0.001). There was no significant difference thigh muscle mass (total or %) between the selected groups at cull. Gp B birds had significantly less total ($p<0.05$) and % ($p<0.01$) thigh muscle mass than Gp D birds.

Total leg bone mass (absolute and %)

All groups had significantly different leg bone masses (total and %) at cull:
In terms of % leg bone mass, Gp C >Gp A > Gp D > Gp B all at $p<0.001$. The same relationship was seen with absolute leg bone mass, except that Gp A > Gp D at ($p<0.01$)

Ratio of breast / thigh muscle

There was no significant difference in the mean ratio of breast to thigh muscle in birds of the same strain at cull. Gp A birds had significantly higher mean ratios of breast to thigh muscle than Gp B ($p<0.01$) and Gp D ($p<0.05$). Gp C birds had significantly higher mean breast to thigh muscle ratios than Gp B and D at $p <0.001$.

Hock Scores

Gp A birds had significantly higher mean hock scores than the other three groups ($p<0.001$). There were no significant differences in mean hock score between the other three groups.

TABLE 4.5: Cull Data – individual leg bone masses, lengths and diameters.

		Gp A	Gp B	Gp C	Gp D	Max. SE of differences
Total femur mass (kg)	**Median** (# SE median)	**0.0267** (0.0004)	**0.0247** (0.0005)	**0.0345** (0.0006)	**0.0279** (0.0005)	0.0275
Total tibiotarsal mass (kg)	**Median** (# SE median)	**0.0415** (0.0007)	**0.0346** (0.0007)	**0.0484** (0.0010)	**0.0376** (0.0008)	0.0308
Total tarsometatarsal mass (kg)	**Median** (# SE median)	**0.0220** (0.0004)	**0.0157** (0.0003)	**0.0224** (0.0004)	**0.0174** (0.0003)	0.0276
Total leg length (m)	**Median** (# SE median)	**0.266** (0.002)	**0.307** (0.002)	**0.319** (0.002)	**0.316** (0.002)	0.0107
Femur length (m)	**Median** (# SE median)	**0.093** (0.0007)	**0.099** (0.0009)	**0.106** (0.0009)	**0.104** (0.0010)	0.0131
Tibiotarsal length (m)	**Median** (# SE median)	**0.116** (0.0007)	**0.139** (0.0010)	**0.142** (0.0011)	**0.140** (0.0011)	0.0110
Tarsometatarsal length (m)	**Median** (# SE median)	**0.058** (0.0005)	**0.069** (0.0007)	**0.071** (0.0007)	**0.071** (0.0008)	0.0152
Tarsometatarsal diameter (lat)	**Median** (# SE median)	**0.0135** (0.0001)	**0.0130** (0.0001)	**0.0147** (0.0002)	**0.0142** (0.0002)	0.0166
Tarsometatarsal diameter (CrCd)	**Median** (# SE median)	**0.0154** (0.0002)	**0.0150** (0.0002)	**0.0159** (0.0002)	**0.0162** (0.0002)	0.0184

Total femur mass

There was no significant difference between median femur mass in Gp A and Gp D birds. Gp C > Gp A / B / D at $p<0.001$, Gp A > Gp B at $p<0.01$, and Gp D > Gp B at $p<0.001$.

Total tibiotarsal (tt) mass

There were significant differences in tibiotarsal mass between all the groups: Gp C > Gp A (at $p<0.001$) > Gp D (at $p<0.01$) > GpB (at $p<0.01$)

Total tarsometatarsal (tmt) mass

There was no significant difference in median tmt mass between the selected groups at cull: Gp A / C > Gp D (at $p<0.001$) > Gp B (at $p<0.001$)

Leg length

There was no significant difference in total median leg length between Gp C and Gp D birds, however Gp C / D > Gp B (at $p<0.01$) > Gp A (at $p<0.001$).

Individual leg bone lengths

Median femur, tibiotarsal and tarsometatarsal lengths of birds in Gp's C, D and B were all greater than those of Gp A birds at $p<0.001$. There were no significant differences in length of the three bones between Gp C and D birds at cull. While Gp B birds did not have significantly different median tarsometatarsal length from the Gp C and D birds, their median femur length was significantly shorter ($p<0.001$) than those of the Gp C and Gp D

birds, and their median tibiotarsal length was significantly shorter than the Gp C birds ($p<0.05$).

Tarsometatarsal diameter - lateral

There were significant differences between all groups at cull weight:

Gp C = Gp D > Gp A (at $p<0.01$) > Gp B (at $p<0.001$).

Tarsometatarsal diameter – craniocaudal (CrCd).

Gp A birds median CrCd tmt diameter at cull was not significantly different than the GpB or C birds, but was significantly less than Gp D birds at cull ($p<0.01$). Gp B birds median CrCd tmt diameter was significantly less than Gp C ($p<0.01$) and Gp D birds at cull ($p<0.001$). There was no significant difference between Gp C and Gp D birds at cull.

TABLE 4.6: Cull data – bone mineral values.

		Gp A	Gp B	Gp C	Gp D	Max. SE of differences
% ASH	**Mean** (SE)	**42.08** (1.431)	**47.34** (1.632)	**52.94** (1.632)	**59.36** (1.720)	2.433
% Calcium	**Mean** (SE)	**20.34** (0.673)	**21.03** (0.767)	**22.00** (0.767)	**24.65** (0.809)	1.144
% Phosphorus	**Mean** (SE)	**7.44** (0.248)	**8.23** (0.283)	**8.36** (0.283)	**9.58** (0.298)	0.422
Calcium / Phosphorus ratio	**Mean** (SE)	**2.746** (0.065)	**2.557** (0.074)	**2.617** (0.074)	**2.579** (0.078)	0.1108

% ash

There were significant differences in % ASH between all the groups:

Gp D > Gp C (at $P<0.05$) > Gp B (at $p<0.05$) > Gp A (at $p<0.05$).

% calcium (%Ca)

Gp D birds had significantly higher mean %Ca levels than Gp A birds ($p<0.001$), Gp B birds ($p<0.01$), and Gp C birds ($p<0.05$). There was no significant difference between the other 3 groups.

% phosphorus (%P)

Gp D birds had significantly higher mean %P than the Gp A birds ($p<0.001$), and the Gp B and C birds ($p<0.01$). Gp A birds had significantly lower mean %P than Gp C birds ($p<0.05$) and Gp D ($p<0.001$). There was no significant difference in mean % P at cull between Gp B and Gp C birds, or between Gp A and Gp B birds.

Calcium / Phosphorus ratio (Ca/P ratio)

There was no significant difference between the Ca/P ratio in any of the groups at cull.

Table 4.7: Cull data - percentage of total leg bone length and mass made up by each individual bone (plus absolute totals, and age in weeks).

		Femur %	Tibiotarsus %	Tarsometa-tarsus %	Absolute Total	Age (weeks)
Length	**GpA**	34.9	43.4	21.7	0.267 kg	6
	GpB	32.2	45.1	22.6	0.307 kg	12
	GpC	33.2	44.6	22.2	0.319 kg	13
	GpD	33.1	44.4	22.5	0.315 kg	24
Mass	**GpA**	29.6	46.1	24.3	0.090 m	6
	GpB	33.0	46.1	20.9	0.075 m	12
	GpC	32.8	45.9	21.3	0.105 m	13
	GpD	33.6	45.4	21.0	0.083 m	24

Table 4.7 shows the median leg bone proportions for the groups. The leg bone proportions are quite consistent between the Gp B, C and D birds, with age appearing to have no effect. In contrast, the Gp A birds appear to have a smaller percentage of their total leg bone mass made up by a longer femur, and a greater percentage made up by a shorter tarsometatarsus.

Table 4.8 (a): Scaling factors (regression coefficients) of body measurements on bodyweight up to 6 weeks of age only (*at 19 degrees of freedom)

Variable		deviance*	Gp A	Gp B	Gp C	Gp D	max SED
girth	l^2	43.03	0.43	0.38	0.43	0.38	0.02057
leg length	l	405.7	0.46	0.49	0.55	0.51	0.02368
thigh circumference	l^2	57.66	0.39	0.37	0.43	0.37	0.03218
tmt width -CrCd	l	84.41	0.32	0.34	0.37	0.35	0.01933
tmt width -lat	l	86.96	0.32	0.31	0.38	0.35	0.01959

Table 4.8 (b): Scaling factors (regression coefficients) of body measurements on bodyweight up to cull weight (*at 16 degrees of freedom)

Variable		deviance*	Gp A	Gp B	Gp C	Gp D	max SED
girth	l^2	52.24	0.43	0.39	0.45	0.39	0.01298
leg length	l	518.1	0.46	0.43	0.50	0.42	0.01769
thigh circumference	l^2	172.7	0.39	0.46	0.53	0.51	0.02611
tmt width -CrCd	l	99.44	0.32	0.32	0.35	0.35	0.01218
tmt width -lat	l	93.27	0.32	0.31	0.38	0.35	0.01193

Tables 4.8a and **4.8b** show the results of regression of body measurements using REML. In isometry, the length measurements should scale to (bodyweight) $^{0.33}$ which is approximated by the tarsometatarsal measurements, but exceeded by the leg lengths, which show positive allometry. The girth and thigh measurements are circumferences, and so equate to (length) ^{2} and should scale to (bodyweight) $^{0.66}$ in isometry. The results suggest that they scale with negative allometry to bodyweight. These results must be regarded with care however, as it can be seen that the deviance associated with lack of fit to a single straight line is large (statistically significant).

Table 4.9: Tibiotarsal torsion and tibial plateau angle measurements

Bird	Tibial plateau angle		Tibiotarsal torsion				Bird	Tibial plateau angle		Tibiotarsal torsion			
	L	R	L		R			L	R	L		R	
A7L	24	24	12.6	e	7.99	i	**C4R**	22	23	6.17	e	4.95	e
A5L	20	23	6.09	e	8	e	**C1R**	22	18	4.97	i	3.09	i
A6L	25	25	7.15	e	4.31	e	**C5L**	12	13	7.07	e	7.21	e
A6R	24	22	12.3	i	5.96	i	**C5R**	15	12	3.74	e	4.46	i
A2R	22	21	6.25	e	5.83	e	**C4L**	22	24	0	*	2.88	i
A4L	21	25	8.18	i	0	*	**C3R**	16	16	2.89	e	0	*
A1R	33	30	3.46	i	0	*	**C1L**	20	18	6.11	e	7.28	i
A5R	17	18	5.49	i	3.47	e	**C2L**	18	17	3.59	e	4.82	i
A1L	15	18	2.65	i	6.67	i	**C2R**	21	20	7.72	e	10.04	e
A2L	24	25	4.01	i	4.56	e	**C3L**	15	18	4.47	i	6.07	i
A3R	22	25	13.2	e	2.01	i	**C5L**	12	13	5.97	e	5.33	e
A4R	22	26	4.24	i	6.28	i	*	*	*	*	*	*	*
A3L	21	20	3.85	i	4.17	e	*	*	*	*	*	*	*
Mean	***22.3***	***23.2***	***6.9***	*	***4.6***	*	***Mean***	***17.7***	***17.4***	***4.8***	*	***5.1***	*
SD	***4.29***	***3.39***	***3.4***	*	***2.6***	*	***SD***	***3.93***	***3.91***	***2.2***	*	***2.6***	*
B3R	14	16	6.71	e	3.28	e	**D3L**	24	21	3.86	e	5.25	e
B5R	20	16	4.07	e	7.31	e	**D2R**	20	21	6.07	e	5.47	e
B3L	15	15	8.36	e	7.31	e	**D1L**	16	16	10.22	e	3.72	e
B2R	23	20	3.76	e	6.55	e	**D1R**	24	25	4.28	e	4.88	i
B5L	18	19	2.95	e	3.65	i	**D5R**	19	21	0	*	5.58	i
B4L	20	24	2.79	i	2.96	i	**D3R**	18	21	2.76	i	2.33	i
B4R	18	20	4.79	e	4.29	i	**D4L**	22	18	0	*	3.97	e
B1R	23	25	0	*	3.97	i	**D5L**	22	17	0	*	0	*
B1L	22	25	5.46	i	2.74	e	**D2L**	14	19	2.9	i	0	*
B2L	19	17	0	*	2.9	e	*	*	*	*	*	*	*
Mean	***19.2***	***19.7***	***3.9***	*	***6.1***	*	***Mean***	***19.9***	***20.2***	***3.3***	*	***3.5***	*
SD	***3.08***	***3.83***	***2.7***	*	***3.4***	*	***SD***	***3.5***	***2.7***	***3.3***	*	***2.2***	*

e = external rotation i = internal rotation

Table 4.9 shows the raw data of the bone measurements. The tibial plateau angles of two of the selected birds exceeded the maximum of 25 degrees quoted for 'normal' birds (Lynch *et al*, 1992). None of the tibiotarsi showed abnormal degrees of external rotation (i.e.

> 20 degrees), however many showed abnormal internal rotation. The highest number of internally-rotated tibiotarsi occurred in the *ad libitum*-fed selected group of birds.

No evidence of tibial dyschondroplasia was found on histological examination of the proximal tibiotarsal sections.

4. 4 DISCUSSION

A large part of this experiment involved taking measurements from living animals, which is often less accurate than taking the same measurements from *post-mortem* samples. An alternative would have been to use more birds, and to cull some each week from which to take the measurements. Increasing the number of birds in the study would have made it impossible to make all the gait measurements in a single day, however, which would have introduced further variation into the results. Although *in vivo* measurements may be less accurate, the variability introduced by measurement error should be the same for all the birds. A recent study also showed that the variability of morphometric measurements could be reduced to as little as 1% if they are all made by one person (as in the present study), compared to 50% in those made by different people (Burbridge and Pfeiffer, 1998).

Aspects of scaling were investigated between the groups using linear regression analysis, however the initial results suggested that this would not be as straightforward as was initially supposed. The large deviance associated with lack of fit to a single straight line suggests that even if the regression on logweight was statistically significant, it may not successfully explain all the variation in the response measurements between different ages. There may be curvilinearity with logweight, or other unobserved variables may explain part of the variation. Scaling could have been further investigated using advanced statistical methods to fit individual lines to each age, or perhaps by fitting curves, however this is outside the scope of this study at present.

Morphometric measurements during growth:

Modern broilers have been selected to grow much heavier, at a more rapid rate than their predecessors (Lilburn, 1994). Of the *ad libitum*-fed groups in this study, the selected strain took just 6 weeks to reach 2.4kg; the relaxed birds took almost twice as long. Thus the *ad libitum*-fed selected birds increased their bodyweight 7.1 times between 2-6 weeks, far exceeding the 4 fold increase described Buckner *et al* (for New Hampshire chickens), for

example, in 1950. This initial 6 week period was identified as a period of allometric growth in the modern broiler by Lynch *et al*, (1992), although they did not demonstrate how this was calculated.

A large proportion of the bodyweight of the *ad libitum*-fed selected birds in the present study was accounted for by breast (pectoral) muscle, which formed approximately 19% of their bodyweight at cull. A previous study of breast muscle development in Ross strain birds was carried out by Acar *et al*, (1993). Although this paper did not give the birds' actual bodyweights alongside their muscle mass, it could be estimated from the bodyweight graphs that approximately 12% of the bodyweight of the 6 week old birds was breast muscle mass. A considerable increase in breast muscle mass has therefore been achieved by selective breeding over the last 5 years.

In general, the pectoral muscle makes up about 15% of the body mass in birds, although being the main flying muscle, this varies with activity e.g. hummingbirds have 25-30% of their body mass as pectoral muscle (Greenewalt, 1962, quoted in Schmidt-Nielsen, 1975). While the breast muscle mass of the relaxed birds in this study was around that of the 'bird average' of 15%, the selected birds had 19-20% of their body mass as breast muscle. The increased muscle tissue in the *ad libitum*-fed selected birds is often affected by pathology however (Mitchell, 1999), and these birds cannot fly. This is an unusual situation, as most birds which loose the ability to fly also show a reduction in pectoral muscle (and a relatively smaller keel bone) e.g. ostriches (Raikow, 1985). It is also interesting that the Gp D birds had higher % (though not total) breast muscle mass than the *ad libitum*-fed birds of the same strain. This suggests that the pectoral muscles in these relaxed birds are highly conserved, and the birds put energy into maintaining them, at the expense of the rest of the body. It is also interesting to note the disproportionate increase in breast muscle compared to thigh muscle resulting from selection. The ratio of breast to thigh muscle was significantly different between the strains, but not within the strains (selected birds 1.7-1.8, relaxed birds 1.53-1.55). This disproportionate increase in bodyweight and breast muscle vs leg muscle has been described by other authors in broilers (Lilburn, 1994) and turkeys (Nestor *et al*, 1985; Nestor *et al*, 1987).

It is difficult to estimate breast muscle mass *in vivo* as girth measurements also include the body cavity. As expected, the *ad libitum*-fed selected birds had significantly greater girths than the other three groups up to six weeks. It was surprising to find no significant difference in girth between the *ad libitum*-fed selected birds and either of the relaxed groups at cull, however, despite the higher genetic potential for breast muscle development in the selected strain. The restricted-fed selected birds did have larger girths at cull, however, which could reflect the higher genetic potential for growth, coupled with their longer life span (due to their slower growth rate on the restricted feeding regime). Thus the

relationship between girth and breast muscle mass is not straightforward. The selected birds had significantly higher total and % breast muscle masses than the relaxed birds at the same end bodyweight, reflecting the selection criteria, even although the end girths of the *ad libitum*-fed selected birds were no different. Girth measurements record the circumference of the chest, however, and if the breast muscle develops in an anterior direction, rather than laterally, this would explain an increase in breast muscle without an accompanying increase in girth.

From a biomechanical aspect, developing large amounts of muscle mass anteriorly would displace the CG in the same direction (in contrast to it developing laterally, which would keep the CG in the same place). In contrast to the standing human, where the CG is located just anterior to the second sacral vertebra, the horizontal orientation of the avian vertebral column results in the CG in birds being located well forward of the hip (Gatesy and Biewener, 1991). Work by Abourachid (1993) on giant turkeys showed that selected pectoral hypertrophy displaced the CG anteriorly in comparison to smaller birds, and it seems likely that this is also the case in the *ad libitum*-fed selected broilers. The effects of being 'front-heavy' on gait patterns will be discussed later.

The rapid growth in bodyweight and girth in the *ad libitum*-fed selected birds was accompanied by a rapid growth in leg length, so that these birds had the longest legs of all the birds up to 6 weeks of age. Bone lengths therefore appear to scale isometrically, length slightly more than doubling in a period in which the bodyweight increased about 7 fold (as length α volume $^{0.33}$). The *ad libitum*-fed relaxed and restricted-fed selected birds show approximately a three fold increase over average 12 weeks, for a similar 7 fold increase in bodyweight, suggesting negative allometry. The selected strains had longer legs than the relaxed strains at the same age, however by the same end bodyweight, the *ad libitum*-fed selected birds had the shortest legs, and the restricted-fed selecteds the longest legs of all the groups. This tends to suggest that the selected birds have a higher genetic potential for bone growth than their predecessors (in line with recent selection criteria). Previous work has shown that birds raised on a lower plane of nutrition have longer bones, at the same bodyweight, than birds fed on a higher plane of nutrition (Wagner, 1979). In the present study, the *ad libitum*-fed relaxed birds ate less than the *ad libitum*-fed selected birds and had longer legs at cull (in agreement with Wagner's findings). However, the restricted-fed relaxed birds, which were on the lowest plane of nutrition of all the groups, had shorter legs than the *ad libitum*-fed relaxed birds. This could be explained if the level of nutrition of the restricted-fed relaxed birds was just sufficient to maintain growth, but at well below potential.

Comparisons of morphometric measurements at the same bodyweight:

When investigating the effects of morphology on gait, it is important to consider the relationship between leg muscle and leg bone mass during growth. There was no significant difference in the % thigh muscle mass between the selected groups at cull, however the selected birds had significantly more thigh muscle mass than the relaxed birds. Thus selective breeding for muscle mass has increased both the breast and thigh muscle (though the breast muscle to a much greater extent). This was also reflected in the leg bone masses, the selected strains having greater leg bone mass than the relaxed strains. Within the strains, however, the restricted-fed birds had more leg bone mass than the *ad libitum*-fed birds. This is in agreement with the results of other studies, which have shown that slower growth allows the more slowly maturing skeletal system to 'catch up' with the muscular system (Frost, 1997). The restricted-fed birds would of course have been older by the time they reached the same cull bodyweight, however only the restricted-fed relaxed birds in this study had reached full skeletal maturity (at around 134 days - Reiland *et al*, 1978). As leg bone mass would be expected to increase up to skeletal maturity, it is surprising to find that it is greater in the *ad libitum*-fed selected birds than the restricted-fed relaxed group. This is further evidence that selective breeding is having an effect on increasing leg bone mass. The increase in leg bone mass that accompanies the increase in muscle mass in all the groups should have a positive effect on walking ability. In isometric subjects, volume scales directly to weight, and so muscle moments would be proportional to muscle mass. Assuming the muscles work properly, an increase or decrease in muscle mass will result in an increase or decrease in bone mass (Wagner, 1979 quoting Doyle *et al*, 1970). This is reflected in the results of the present experiment, where the increase in muscle mass is accompanied by an increase in bone mass (even though other studies suggest that muscle function is poor in modern selected birds (Mitchell, 1999)). The birds used in the present experiment therefore differ from those used by Emmerson *et al*, (1991) who showed that selection for increased bodyweight produced consistent increases in muscle mass (high genetic correlation) and negligible changes in leg bone mass (low genetic correlation between bodyweight and skeletal elements).

In terms of biomechanics, the relationship between muscle and bone mass is important since other than the effect of bodyweight, the main forces acting on the bones are produced by the muscles which attach to them. As the peak force that a muscle can generate is proportional to its cross-sectional area, the moment produced by a muscle is therefore the product of its area and length, which in turn is the muscle volume (Van der Mulen *et al*, 1993). The heavier bodyweight of the *ad libitum*-fed selected birds will result in greater

forces being required to overcome inertia, but this is made difficult by the shorter legs (moment arms). Thus larger forces will need to be generated by the muscles during walking in these birds, in comparison to the longer-legged restricted-fed birds, for example, and these large forces will be acting on bones which are still immature.

The results so far fit with the hypothesis that the biomechanical stresses on the musculoskeletal system are different between the strains. The *ad libitum*-fed selected birds in particular become heavier at a younger age, when their bones are still immature. At the same bodyweight, they have shorter legs with larger muscle masses, which would theoretically have to exert greater forces on the limb bones to move the body around. The next stage, therefore, is to consider how the skeletal system might respond to the increased demands being placed on it.

Bone development:

The leg bones of the chicken are obviously different to most mammals, the tibiotarsus being composed of the fused tibia and proximal row of tarsal bones, and the tarsometatarsus being made up of the fused 2nd, 3rd and 4th metatarsals, and the distal tarsal bones (Raikow, 1985). Comparing the ratios of the long bones in the chicken (femur-tibiotarsus-tarsometatarsus) with the equivalent bones in mammals (femur-tibia-longest metatarsal), the values for the chicken (1 : 1.3 : 0.7) are closer to those of horses and ruminants (1 : 1 : 0.7) than cats and dogs (1 : 1 : 0.4) (Alexander, 1977). The massive elongation of the metatarsal lightens the limb, and is usually considered to be an adaptation for speed, at the expense of stability (Merkens, 1987). It is interesting that chickens have taken this developmental path: while it may be useful to reach great speeds prior to being able to take-off, it is likely that the greatest biomechanical benefit comes from the longer lever arms created by the elongated tibiotarsus and tarsometatarsus. Combined with joint flexion, this would enable much greater propulsive forces to be generated for take-off, and also to absorb the 'shock' of landing. This was demonstrated in a cleverly devised experiment, which used a force-transducing perch to measure leg thrust forces in Starlings (Bonser and Rayner, 1996).

The *ad libitum*-fed selected birds had shorter and lighter femurs than the other groups, but heavier tarsometatarsi, suggesting a differential rate of growth between the leg bones. Differential growth rates in leg bones have been shown in other studies: between 2-10 weeks, the leg bones were found to increase 3x in length, and the weight of the bones increased 25 fold (tibia), 27 fold (femur) and 34 fold (metatarsus) (Buckner *et al*, 1950). In

the present study, the tibiotarsus was the longest and heaviest of the leg bones in all the groups, confirming the findings of other studies (Church and Johnson, 1964)[1]. The rapid increase in volume of the tibia indicates a very rapid bone turnover, and increased 'plasticity', of the skeleton of the broiler (Reiland *et al*, 1978). This is beneficial if the stresses are 'normal', but may cause the bones to develop abnormally if the stresses are excessive, which could explain why valgus angulation of the intertarsal joint, with lateral twisting of the distal tibiotarsus, is the most common deformity in broilers (Duff and Thorp, 1985b). In complete contrast, Lilburn (1994) states that relative tibial development is significantly slower in broilers compared to ducks and turkeys, and makes the surprising comment that this would explain its greater susceptibility to biomechanical problems. This seems unlikely.

If we accept the hypothesis that the bones of the modern broiler are subject to greater stresses, we must then consider how they have developed in response to the demands being placed upon them.

Factors affecting bone strength:

Bone strength depends on a number of factors, two of the most important being cross-sectional area and mineral content. Cross-sectional area has been compared to length to produce various measures of bone 'robusticity' (Sumner and Andriacchi, 1996), as has bone weight e.g. Ponderal Index: bone length / $\sqrt[3]{}$bone weight (Simon *et al*, 1984). In the present study, tarsometatarsal diameter was used as a measure of the cross-sectional area of the bone. It is acknowledged that this may not be representative of the true cross-sectional area, if the width of the cortices vary between the groups. The main difference in diameter between the tarsometatarsi of the different groups was in the lateral direction, with differences in the craniocaudal direction being less marked. The selected birds had wider tarsometatarsal diameters than the *ad libitum*-fed relaxed birds, suggesting that their bones were developing in a way that should help to reduce the stresses on the skeleton. The fact that the slower growing restricted-fed birds had wider tarsometatarsal diameters than the *ad libitum*-fed birds in both strains is also important. This occurs because bone development generally lags behind the rest of the body during periods of rapid growth, only catching up when the growth in muscle strength and bodyweight tend to plateau at around skeletal maturity (Frost, 1997). A study of tibial development in poultry, for example, showed that

[1] Church and Johnson (1964) and many other authors have studied the tibia – which is the tibiotarsus with the tarsal bones removed. As the tibia makes up the majority of the tibiotarsus, however, it is likely that comments relating to the tibia are equally applicable to the tibiotarsus.

although longitudinal bone growth appeared to be complete at 92-113 days of age, the weight, volume and density of the bones continued to increase after the longitudinal growth had ceased (Reiland *et al*, 1978). This may be either an adaptation to weight-bearing, or because the rate at which the cross-sectional area can increase is inherently limited (resulting in it requiring time to 'catch up') (Sumner and Andriacchi, 1996). A consequence of bone development lagging behind the increase in bodyweight is that peak strains can often be higher during growth than in adulthood (despite the lower bodyweight) e.g. Frost (1997) quotes peak strains in rapidly growing animals to be between 2000-4000 microstrains, compared to 800-1200 microstrains in adults. Slowing the growth rate of the birds by restricting their food intake appears to allow the bones to develop in a way which would improve the strength of the skeleton, and so should decrease the damage caused by the peak microstrains.

The second feature that can influence bone strength is the mineral content. An increase in the mineral content of bone by only a few percent results in an increase in maximum and yield stress (and modulus of elasticity), and therefore its ability to resist the demands placed upon it (Currey 1975, Currey, 1988, Rose *et al*, 1996). In the present study, the % ash, calcium and phosphorus all increased with age in the birds, as would be expected. Recent work in poultry has shown that poor quality cortical bone (assessed histologically) also had the lowest ash content (Thorp and Waddington, 1997). The 35-day old broilers in the Thorp and Waddington study had ash levels of 47.7%, which is similar to the *ad libitum*-fed relaxed birds in the present study (but less than the two older groups). The *ad libitum*-fed selected birds, however, had 5.62% less ash than those in Thorp and Waddingtons study, which suggests that their bone quality would be poor. The Ca/P ratios of the birds in the present experiment were not significantly different between the groups, however, ranging from 2.5 – 2.7 : 1, which is higher than the normally quoted ratio of 2 : 1 (Thorp and Waddington, 1997). The effect of the altered ratio on the bone is unknown, however Thorp and Waddington (1997) suggest that it may disrupt the structure of the hydroxyapetite crystal, weakening the bone.

On the basis of % ash, the *ad libitum*-fed selected birds had the poorest quality bone of all the groups, despite carrying around a similar bodyweight. However, they also had the widest tarsometatarsi, and other studies have suggested that the increased resistance to bending which occurs during growth is a result of a change in the geometric properties of the bones, rather than the material properties (Sumner and Andriacchi, 1996). It would be difficult to prove that one had a more important role than the other, however.

The studies on bone ash, calcium and phosphorus were based on cortical sections taken from the mid-shaft tibiotarsus, and it is possible that samples from the other bones, or

different parts of the same bone, may have produced different results. Independent mineralisation characteristics of the bones have been demonstrated in other studies (Buckner *et al*, 1950; Itoh and Hatano, 1964; Dilworth and Day, 1965 quoted in Lilburn, 1994).

Having considered the morphometric measurements and conformation of the different groups of birds, the second part of this discussion will describe the gait patterns, and how they relate to the conformation.

Development of gait patterns during growth:

The theory that the increased growth rates and bodyweight of modern poultry have a detrimental effect on locomotor ability has been difficult to prove conclusively. Many studies have shown an increase in leg problems in rapidly growing birds (Nestor *et al*, 1985; Nestor *et al*, 1987; Leterrier and Nys, 1992), while others have found no difference in incidence based on growth rate or feeding regime (Haye and Simons, 1978; Cook *et al*, 1984; Duff and Hocking, 1986). However, it is generally accepted that slowing the rate of weight gain in the early stages appears to allow for more skeletal growth as opposed to muscular development, and this should improve walking ability (Hester *et al*, 1990; Hester, 1994, Lilurn, 1994). The hypothesis that growth rate affects walking ability can be tested by comparing the gait of the *ad libitum*-fed and restricted-fed birds in this study.

The morphometric measurements over the first 6 weeks showed that the rapidly growing *ad libitum*-fed selected birds developed longer legs, which should enable them to take longer steps, and so move faster. Their excess breast muscle development and larger girths resulted in a conformation which should cause the steps to be wider than those taken by the other groups. In fact, the *ad libitum*-fed selected birds in the present study walked at significantly slower speeds than the other birds at all stages during growth. Within strains, the restricted-fed birds moved more quickly than the *ad libitum*-fed birds, possibly because they were lighter, and more highly motivated to look for food. As the restricted-fed selected birds showed similar speeds to the *ad libitum*-fed relaxed birds throughout growth, the fact that these groups were approximately matched for bodyweight suggests that speed in the restricted-fed birds probably has more to do with bodyweight than urgency to find food. Comparing speeds with those found in the previous experiment on Brown Leghorns (0.1m/s) however, it is surprising to find that the *ad libitum*-fed selected birds were the only group which moved at similar speeds, the other 3 groups all being faster. The most likely explanation for this is that the broilers were more familiar with the testing procedure, and more highly motivated to rejoin their group at the end of the runway. In the Brown Leghorn

experiment, the birds were more cautious, and had less motivation to walk. This illustrates the difficulties of comparing absolute values obtained in different experiments where conditions have varied, and also the important role which motivation appears to play in birds' locomotor performance. Other studies, such as Wetzel and Stuart (1977) have shown that both spatial and temporal aspects of gait are altered depending upon whether the stimulus to walk is pleasant e.g. a food reward, or aversive.

The results of the present study also showed that speed was very variable, with no clear trend in any of the groups as the birds grew. This is in contrast to other studies, where maximal speed was found to increase with age from 0.48-1.17 m/s (Biewener *et al*, 1986). The aforementioned study was performed on birds which were trained to run on a treadmill, however, and it is accepted that subjects will alter their gait when tested on a treadmill (Leach, 1987, quoting Fredricson *et al*, 1983).

Speed is a function of step length and step frequency, therefore each needs to be considered in turn. It would be expected that birds would take longer steps as their leg length increased, and this was confirmed: step length showed a significant increase during growth in all groups. Despite having longer legs at the same age, however, the *ad libitum*-fed selected birds took significantly shorter steps (0.097m) compared to the other groups, including the Brown Leghorns of the previous study (0.11m). The other 3 groups took considerably longer steps (0.160 - 0.209m).

It is interesting that the *ad libitum*-fed selected birds, which had the longest legs for the first 6 weeks, actually took the shortest steps, and moved most slowly. The results of the Brown leghorn work showed that at low speeds (as seen in the present experiment), speed was influenced more by cadence than step length. In theory, leg length should affect step length more than cadence, and so this might explain why the extra leg length of the *ad libitum*-fed selected birds did not affect their speed. Increased leg length usually results in increased step or stride length, however (Sutherland *et al*, 1988), and so we must consider why the *ad libitum*-fed selected birds take shorter steps. It may, at least in part, be due to the increased breast muscle displacing the CG anteriorly, which would result in the feet having to be placed slightly further forward under the body for support. This would presumably be achieved mostly by flexion of the hip joint, which would be limited, and so the CG may only just fall within the area of support provided by the feet. As soon as the bird moves forward, the CG would fall outside of the area of support, and the other foot would have to be quickly replaced to re-establish the equilibrium.

As discussed in the introduction, the main aim of locomotion is to propel the CG forwards as energy efficiently as possible, while maintaining stability. If we consider stability first, the period when the bodyweight is taken on one leg is the period of greatest

instability. Decreasing step length decreases the swing phase during which time one leg is off the ground. Decreased step or stride length is therefore a commonly used method of improving stability, and is seen in the gait of young children (Todd *et al*, 1989) and elderly men (Murray *et al*, 1969), and also various types of pathological gait (Whittle, 1991). Taking shorter steps is also a more efficient way of walking at slower speeds. Taking long steps at slow speeds results in large excursions of the CG (which is raised when the feet are together, and lowest when the angle between the two legs is greatest) (Cavagna and Margaria, 1966). This 'wastes' energy, as force has to be applied to the ground to raise and reaccelerate the CG with each step.

As speed did not change significantly despite the increase in step length during growth, it was not surprising to find a significant decrease in cadence (in all groups except the restricted selected birds). This is in agreement with various studies on human gait, which show that cadence decreases with age (as stride length increases) (Sutherland *et al*, 1988; Todd *et al*, 1989). These results contrast with the findings of Biewener *et al* (1986) in birds, however, which showed that stride frequency remained constant with age (1.74 +/- 0.06 SD strides / second). Again, these results must be interpreted in context, as the speed of the birds would have been determined by the treadmill, and so should not be taken as being representative of normal walking.

The cadence of the *ad libitum*-fed selected birds was similar to that of the Brown Leghorns in the previous experiment (53.5 steps / minute), which is not surprising as the step lengths and speeds were similar between these groups. The other three groups in the present experiment all showed higher cadences. Reducing cadence is another way of reducing the duration of single support, as a decrease in cadence increases the cycle length mainly by increasing stance phase (and therefore the double support time) (Whittle, 1991).

If we assume that all the birds in the present experiment were equally motivated to walk, the results so far suggest that the *ad libitum*-fed selected birds were prevented from moving more quickly, either because they were physiologically unable to do so, or because of an inappropriate body conformation. The slower speed and shorter steps of the *ad libitum*-fed selected birds could also be a response to instability. In this case, there are a number of other ways in which they could improve their balance, the most effective being to increase their support base. As discussed in the introduction, a subject remains stable as long as the line of force passing vertically down from the CG remains within the area on the ground that is supporting it (Whittle, 1991). Increasing the support base allows for a wider 'margin of error' in positioning the CG, and this can be achieved, for example, by the use of canes and crutches by elderly or disabled people (Whittle, 1991). The results of the present

study suggest that chickens increase their support base in two ways: firstly, by taking wider steps, and secondly by altering the position in which the feet were placed on the ground.

Step width increased significantly as the selected birds grew, in contrast to the relaxed birds, where it did not change significantly. Within the strains, there was no significant difference between the groups, which suggests that growth rate did not have an effect. Step widths in the Brown Leghorns were approximately 0.02 m, similar to the relaxed birds, but the steps taken by the selected birds were almost three times as wide. This is in contrast to various studies, which demonstrated that walking base decreases with age in normal human bipeds (Todd *et al*, 1989; Whittle, 1991). Increases in walking base are reported in elderly subjects (Murray *et al*, 1969), and people with proprioceptive or balance defects, such as arise in cerebellar ataxia and Parkinsonism (Murray *et al*, 1978; Gabell and Nayak, 1987; Cunha, 1988). Various studies on animals have demonstrated that step width increases markedly in lameness, confirming that it is a useful measure of the degree of difficulty in walking (Clarke and Parker, 1986; Sheets *et al*, 1987; Farage-Elwar, 1989).

The increased step width of the *ad libitum*-fed selected birds is strong evidence that they are unstable during locomotion. If it was simply that the expanding girth of the birds was forcing the legs further apart, the other groups would have similar step widths on reaching the same end girths, but this was not the case. Unfortunately, a markedly increased walking base, although improving stability, makes locomotion difficult for other reasons. In order to position the CG over the stance leg, the birds have to tip their body laterally, and so this is another reason why they would keep the single-stance periods as short as possible. Human bipeds with wide walking bases adapt by using lateral trunk bending, which allows the CG to be positioned over the stance leg. This does not appear to happen in birds, however, presumably because their more rounded bodies and fused synsacrums make such bending of the vertebral column impossible. In terms of work done during walking, this type of gait also 'wastes' a lot of energy through having to accelerate the CG in such marked lateral excursions. In human walking, the gait optimisations result in the vertical and lateral displacements being approximately equal (Saunders *et al*, 1953), however this is obviously not the case with the *ad libitum*-fed selected birds. Interestingly, a similar pattern is seen in elderly people, where the vertical movement of the head (reflecting the movement of the CG) is reduced, and the lateral movement is increased (Murray *et al*, 1969). The actual GRF's involved in walking birds will be measured in subsequent experiments.

A second way to increase the support base would be to turn the feet out i.e. adopt a 'toe-out' posture rather than a 'toe-in' one. This would effectively widen the support base by the length of the middle toe, increasing the lateral support (almost like having stabilisers on a bicycle). While the step angle was very variable during growth, the most obvious

difference was that the *ad libitum*-fed selected birds pointed their toes outwards, while all the other groups pointed them inwards. The relaxed birds and the restricted-fed selected birds all showed lower degrees of inward turning of the foot (1.6-20.1 degrees), than the Brown Leghorns (average 33.3 degrees). This indicates that all the birds in the present experiment increased their support base compared to the Brown Leghorns, suggesting a degree of instability in all the broilers. The fact that the restricted-fed selected birds feet turned inwards could indicate that growing more slowly reduces locomotor instability. Turning the feet outward may be a method that birds use to make up for their inability to bend their trunks laterally. As discussed earlier, improving stability by developing a wide walking base results in larger lateral excursions of the CG, which increases the inefficiency of walking. Turning the foot outward, however, will increase the walking base / lateral support without requiring the CG to move any further out, as it remains above the actual leg. An increased angle of 'toe-out' has also been reported in the gait of elderly people (Murray *et al*, 1969). A study by Sheets *et al* (1987) also showed that the foot was placed with the 3rd (medial) toe pointed inwards ('toe-in'), in normal chicks, but found that in lame birds, the feet pointed more in line with the direction of walking (effectively turned outwards). External rotation of the leg is also a method used in human walking to compensate for quadriceps weakness, to change the direction of the line of force through the knee, and facilitate hip flexion (by using the adductor muscles, if the true hip flexors are weak) (Whittle, 1991). To point the feet outwards, when it would appear that they would normally point inwards in the chicken, must have consequences on the skeletal system, not least of which would involve rotation at some point in the leg. This will be discussed further in the section on pathology.

So far, the results support the hypothesis that the *ad libitum*-fed selected birds are attempting to increase their stability by walking more slowly, taking shorter steps and increasing their walking base. The next stage is to examine how this is reflected in the gait cycle.

Gait cycle:

As timing of the phases of the gait cycle change with cycle length (and therefore speed), comparisons can be most accurately made by comparing the events as percentages of the total cycle time. The % stance, % swing and % double contact times are all inter-related, and none changed significantly during growth in any of the groups.

In general, the *ad libitum*-fed selected birds had significantly longer stance times than the relaxed birds, but no different from the restricted-fed selected birds. The longer stance periods and shorter swing periods mean that the foot spends longer on the ground

(i.e. has a higher duty factor), giving better stability. During growth, the trend in % double contact time was for the *ad libitum*-fed selected birds to be highest, followed by the restricted-fed selected birds, and then the relaxed groups to be more variable. In 'normal' human walking, the stance phase usually accounts for approximately 60% of the gait cycle, swing phase for 40% (Whittle, 1991), In this study, only the restricted relaxed birds approximated to this during growth (36 - 65%); the other groups were very different, with much higher % stance times. The *ad libitum*–fed selected birds averaged above 80% from the second week on to cull, ending up at 82% stance, and 18% swing. In humans, the % double contact periods are approximately 10% for each foot (Whittle, 1991), which is the same as the restricted-fed relaxed birds (20% for both feet), but far less than the 35 % for the *ad libitum*-fed selected birds.

Again, the gait patterns of the *ad libitum*-fed selected birds showed similarities to those of less stable humans i.e. children and the elderly, both of which use shorter swing phases and/or longer stance and double contact periods (Murray *et al*, 1969, Todd *et al*, 1989). There have been few other studies on birds that have measured cycle timing. A study by Jacobson and Hollyday (1982) reported absolute value ranges: stance phase 220 -970 ms, swing phase 70-240 ms and total cycle duration 330 -1,050 ms. As the times will vary depending on the speed however, it is difficult to make useful comparisons with these results.

The longer stance and double contact times, and shorter swing periods of the *ad libitum*-fed selected birds compliment previous findings in supporting the hypothesis that these birds are attempting to improve their stability. In particular, the longer double contact periods mean that there is a wider support base for a greater time.

There are also other benefits to keeping the foot in ground contact longer. The morphological study showed that these birds were moving larger masses on relatively shorter lever arms (composed of immature bone), and therefore the skeleton would be subject to greater stresses. Assuming a given load has to be moved, the total force exerted by a limb (the 'impulse') = force x time. So for a given impulse, increasing ground contact time enables the peak forces to be reduced, decreasing the strains on the bones, helping to keep them within 'safe limits'. As discussed in the general introduction, exceeding 'safe limits' can cause excessive microdamage, which can result in pathological remodelling and eventually failure of the bone (Rubin and Lanyon, 1985; Frost, 1994a,b). The period of single leg contact is the period of greatest stress, when the whole bodyweight (and mass of the other leg) is taken by the single leg. Various studies on unilateral weight-bearing in poultry have reported an increased incidence of abnormalities such as dyschondroplasia in

the weight-bearing or 'over-loaded' limb (Duff, 1986; Thorp and Duff, 1988). It is therefore beneficial to share the load between the two legs for as much time as possible – hence the increased double contact times.

Pathology:

The hock scores showed that all groups had lesions suggestive of some degree of inflammation (redness and or swelling). The scores were significantly higher in the *ad libitum*-fed selected birds, with mean values greater than 3, indicating significant skin bruising in these large heavy birds, which seemed to spend most of the time sitting. The lower hock scores in the restricted-fed selected birds would support the observation that they spent far less time sitting down, as seemed to be the case when the birds were being observed. *Ad libitum*-fed selected birds are known to be relatively inactive, and various studies have shown that increasing the hours of light increases activity, and decreases leg problems (although the intensity of lighting does not matter) (Hester, 1994). It is interesting to consider in this context the point made by Frost (1994b), that 'load without motion or motion without load seldom make them (*damaged joints*) worse, but combining the two usually does'. Thus if the broilers do not have the skeletal system to support their bodyweight during exercise, it may be beneficial to spend long periods sitting. However if the level of activity is too low, the stresses on the bones may be inadequate to maintain normal development, as a certain level of stress is required to produce and maintain adequate bone mass post-natally (Rubin and Lanyon, 1987; Sumner and Andriacchi, 1996).

The bones were also examined *post-mortem* for any evidence of sub-clinical pathology. As the birds were not lame, the examination was limited to 'screening' for the common problems of tibiotarsal torsion and tibial dyschondroplasia. Tibial plateau angles were also measured, to assess tibial bowing, which is often associated with TD (Lynch *et al*, 1992). All of the *ad libitum*-fed selected broilers had valgus angulation of the intertarsal joint (as described by Duff and Thorp, 1985a, b) however the angles were not measured in the present experiment. Valgus angulation is more commonly seen in the knee joint in people, where a mild degree of valgus enables the tibia to be kept vertical while the femur inclines towards the median from a slightly adducted hip; a marked valgus abnormality, however, leads to a pathological gait (Whittle, 1991).

The results show a number of interesting features, although none of the birds were clinically lame, and so all were retained in the study. No evidence of tibial dyschondroplasia was found on histological examination of the proximal epiphyseal tibiotarsal sections, although some birds showed slightly greater tibial plateau angles than is considered normal.

This does not exclude the possibility that dyschondroplasia was present at other sites, however, or that TD lesions had been present, and had subsequently resolved.

None of the birds had an abnormal degree of external rotation of the tibiotarsus, but some had internal tibiotarsal rotation (particularly the *ad libitum*-fed birds). This did not result in gait patterns that deviated from normal however. A study comparing broilers (*ad libitum* and restricted-fed) and laying strain birds found that the tibia was normally rotated externally (up to 20 degrees), however there was a high incidence of internal rotation in the *ad libitum*-fed broilers, which was suggested to be pathological (Duff and Thorp, 1985 a, b). A subsequent publication by Lynch *et al* (1992), quotes a normal range for tibiotarsal torsion as –5 to 20 degrees, making allowance for a mild degree of internal torsion. The results of the present study suggest that the effect of mild internal torsions on gait appears to be insignificant by itself. .

It is interesting that the *ad libitum*-fed selected birds were the only group to point their toes outwards, despite the higher incidence of internal tibiotarsal rotation. Even in birds with external rotation, the degree of rotation seems insufficient to explain the extent to which the foot was turned outward in this particular group. The examination was limited to the tibiotarsus, however, and obviously rotation can occur elsewhere in the limb. The study by Duff and Thorp (1985 a) found that the femur tended to rotate externally, while the tarsometatarsus rotated internally. The question is one of cause and effect: do problems in bone development result in limbs that rotate outwards, or does the requirement to increase stability by increasing walking base produce forces that tend to change the bone growth? The fact that bone morphology is 'sculpted' in response to the functional demands placed on the bone (Bain and Watkins, 1993) tends to suggest the latter. If abnormal stresses are created within the bones due to the birds having to turn their feet outwards for balance (a torquing effect), this will also cause problems in the joints and tendons, which will further compromise walking ability. Further work needs to be undertaken to answer this question.

Summary:

The results of this experiment suggest that the musculoskeletal system of the *ad libitum*-fed selected bird is under considerably greater biomechanical stress than those of the other groups during growth, and this appears to be reflected in the gait pattern. The *ad libitum*-fed selected birds reach heavy bodyweights at a young age, with wide girths and large amounts of breast muscle displacing the centre of gravity anteriorly. They have shorter legs than the other groups, and greater thigh muscle masses (thus greater forces can be exerted by shorter lever arms). The tarsometatarsi are broader, helping to resist the greater

loads, but the bones have lower mineral content, which would theoretically make them weaker.

In terms of gait, the *ad libitum*-fed selected birds walk more slowly, with lower cadence and taking shorter steps. Their steps are wider, and they point their toes outwards to improve their balance. They keep their feet in contact with the ground for longer, having long % stance times, short % swing times and increased double contact times compared to the relaxed birds. These are all ways of improving stability during walking.

This study has shown that the conformation of the birds could be responsible for causing the apparent difficulties in walking. This is without any consideration of the problem of having defective muscles, and a cardiovascular and respiratory system that may not be able to support the physiological demands of locomotion in these birds. While much work is being done on both of these aspects, it is outwith the scope of this thesis.

The question of whether these types of bird are suffering any sub-clinical pain is addressed in a subsequent experiment, presented later in this thesis (Chapter 6).

CHAPTER 5:

A FORCE PLATE STUDY OF THE GAIT OF BROWN LEGHORNS.

5. 1 INTRODUCTION

Previously in this thesis, the pedobarograph was used to measure plantar pressures in Brown Leghorns during walking, and from these, net vertical forces were estimated. Force is a vector quantity, and the net vertical force is the product of the 3 orthogonal ground reaction forces (GRF's). The magnitude and duration of the individual ground reaction forces can be measured using a Kistler force plate.

Ground reaction forces have not been described in detail in chickens, however the vertical force component would be expected to be closely tied to bodyweight, and have characteristics similar to those of other bipeds, such as humans. The craniocaudal and mediolateral forces are more variable, and it will be interesting to see how these compare between species. The ground reaction forces produced during normal walking in Brown Leghorn hens are described and measured in this experiment.

5. 2 MATERIALS and METHODS

Birds

Eighteen five month old Brown Leghorn hens were used in this study: all the birds were normal on clinical examination, and showed no sign of lameness. The birds were initially raised in pairs in mesh-floored battery cages, but were moved to floor pens littered with wood shavings two weeks prior to testing.

A week prior to testing, the birds were taken to Aberdeen, and housed together in a large floor pen ($3.5m^2$), littered with wood shavings, in the same room as the testing would take place. The birds were fed in this pen, but it was not enclosed, and so they were able to leave the pen and explore their environment. The home pen was situated at one end of the testing runway, and a small 'holding' pen built at the opposite end. The birds were fed on Roslin layer pellets (Appendix 5.1), and a feeder and drinker were placed in each pen to encourage the birds to become familiar with the pens and runway set-up.

Room lighting was provided by 3 x 60 W bulbs (on a 14 hours light / 10 hours dark regime), and a 15 W fluorescent strip light was mounted above the force plate to provide additional light for the video cameras. The room temperature was maintained at 20°C, and the humidity maintained at around 50%.

Equipment

The Kistler force plate and associated equipment is described in detail in the Chapter 2.2. In this experiment, the force plate was set at a sampling rate of 100Hz, over a period of 2 seconds.

Testing

The testing set-up is illustrated in Figure 2.6.

The runway was enclosed by a solid wall on one side and a mesh fence on the other. The force plate was positioned in the centre of the runway, with a 1.2 meter board laid at either end, and the whole surface of the runway was made level. Trial runs were performed in the week before testing began, the birds being placed in the 'holding' pen, and encouraged to walk back across the runway to their home pen.

During testing, the birds were selected in random order, and only runs in which the birds crossed the plate in a reasonably straight line, at a steady walking speed, were saved for analysis. The aim was to collect ten runs (five runs from each bird on two consecutive days). Due to a technical problem with the force plate however, the runs could not be collected on the second day. Instead, the birds were re-tested 16 days later, when a further 10 runs were obtained from each bird. The runs from both sessions were included in the analysis, and the possible confounding effect of the time lapse between the different sessions has been taken into account in the statistical analysis of the data.

Gait Parameters

The gait parameters measured in this experiment are described in Table 2.2. The abbreviations used in the tables of results are listed at the start of this thesis (Abbreviations).

Data Analysis: (see Chapter 2.5)

The forces were 'normalised' and expressed as percentages of the birds' bodyweights to enable comparisons to be made between birds of different weights. Measurements made on runs were considered within speed group ranges, and certain measurements made on steps are expressed as a % of the total stance time.

The following speed ranges were used: (see Chapter 2.5 for derivation)

'fast' speed = 0.793 - 1.049m/s

'medium' speed = 0.473 - 0.729m/s

'low' speed = 0.153 - 0.409m/s

The effects of within bird, within run variance were eliminated by considering only one step per run per bird. The data on peak vertical force was found to be much more normally distributed if the bodyweight was subtracted i.e. expressed as %bodyweight-100, so that effectively only the % above bodyweight was used. All measurements except Brake % were assumed to have normal distributions and constant variances in the logarithmic scale, and so were transformed prior to analysis using REML (Patterson and Thompson, 1971) to produce estimates of the means and variances. Brake % was assumed to be normally distributed with constant variances in the percentage scale.

As some of the variance components were estimated (and therefore subject to sampling variation), the 95% ranges for new single transformed observations or bird means of transformed observations were calculated approximately in the logarithmic scale using Students *t* test with 17 degrees of freedom. The results were then back transformed, and multiplied by an empirical adjustment linking the mean of logarithms to the mean of untransformed values.

5. 3 RESULTS

Initially, analysis of the pooled data set was performed, and this was subsequently repeated with the data grouped into the three speed ranges. In all measurements, the mean is greater than the median. This indicates that there are a few very high values 'skewing' the data, and so more useful comparisons can be made using the medians, as this statistic is more resistant to the effects of a few extreme values.

Table 5.1: Pooled data - means, medians and coefficients of variation of ground reaction force measurements, at any speed.

MEASUREMENT	on	CV-1	CV-2	Median	Mean	95 % range for a new observation
Fz max – 100%	run	49 **	44	32.5	36.1	12.2 – 86.4
Fz slope	run	99 **	75	8.4	11.8	1.3 – 54.0
Fy max	run	42 **	36	30.2	32.8	12.3 – 73.6
Fy min	run	42 **	39	25.3	27.4	10.7 – 59.4
Fx max	run	57 ns	54	13.9	16.1	4.5 – 43.6
Fx min	run	64 **	57	13.8	16.3	3.9 – 48.3
Y ratio	run	67 **	59	1.19	1.14	0.31 – 4.55
X ratio	run	69 ns	67	1.01	1.23	0.27 – 3.77
Speed	run	37 *	34	0.56	0.593	0.262 – 1.184
Stance time	step	33 *	30	0.32	0.336	0.162 – 0.628
Brake %	step	28 ns	26	52.2	52.2	21.6 – 82.8
Braking rate	step	76 ns	70	1.6	2.02	0.38 – 6.73
Propulsion rate	step	113 *	102	6.55	9.86	0.93 – 46.03
Braking integral	step	107 *	94	0.0155	0.0227	0.0024 – 0.0987
Propulsion integral	step	88 ns	85	0.0154	0.0205	0.0031 – 0.0766

CV-1 = coefficient of variation between runs from different birds and sessions

CV-2 = coefficient of variation between runs from same bird and session

Bird or session component of variance statistically significantly different from zero:

** $p < 0.01$ * $p < 0.05$ ns $p > 0.10$

Table 5.1 shows that the coefficients of variation are very high indicating that the measurements vary considerably between different birds and sessions, and even between runs made by the same bird during a single session. The 95% ranges for a new observation are therefore large. Stance time and brake % have relatively lower coefficients of variation. As the brake % equals 100 - % of stance time spent in propulsion (prop %), the % time spent in either braking or propulsion during a single stance period appears to be approximately equal. The median braking and propulsion integrals are almost equal as might be expected from the equal % time spent in each phase, however the median propulsion rate is approximately 4 x the median braking rate.

Table 5.2: Pooled data - 95% ranges for a newly observed bird mean, at any speed

MEASUREMENT	on	Number of runs per bird :		
		5	10	15
Fz max – 100%	run	19.3 – 61.1	21.0 – 57.9	21.8 – 56.7
Fz slope	run	2.5 – 38.3	2.7 – 36.7	2.8 – 36.2
Fy max	run	17.2 – 57.7	18.2 – 55.6	18.5 – 54.8
Fy min	run	16.2 – 43.5	17.5 – 41.0	18.1 – 40.2
Fx max	run	8.1 – 28.2	9.1 – 26.1	9.6 – 25.4
Fx min	run	7.0 – 32.4	7.8 – 30.4	8.1 – 29.7
Y ratio	run	0.56 – 3.12	0.61 – 2.93	0.64 – 2.88
X ratio	run	0.59 – 2.16	0.71 – 1.96	0.76 – 1.85
Speed	run	0.383 – 0.875	0.413 – 0.826	0.424 – 0.808
Stance time	step	0.223 – 0.484	0.237 – 0.462	0.242 – 0.454
Brake %	step	35.5 – 68.9	38.2 – 66.2	39.2 – 65.2
Braking rate	step	0.83 – 4.04	0.97 – 3.68	1.03 – 3.57
Propulsion rate	step	2.6 – 25.3	3.3 – 23.2	3.5 – 22.5
Braking integral	step	0.0064 – 0.0566	0.0076 – 0.0514	0.0082 – 0.0500
Propulsion integral	step	0.0084 – 0.0407	0.0103 – 0.0354	0.0113 – 0.0336

Table 5.2 shows that the 95% ranges are large, as would be expected from the high coefficients of variation seen in Table 5.1. Increasing the number of runs used would not reduce the ranges by very much, however.

It was thought that the differences in speed of the runs may be causing the high variability seen in these results, and so it was decided to re-analyse the data within defined speed ranges and test if speed has an effect on the medians.

Table 5.3: Data within speed ranges: means, medians, coefficients of variation and 95% ranges for ground reaction force measurements made on runs.

MEASUREMENT	CV - 1	CV - 2	Speed range	Median	Mean	95 % range for a new observation
Fz max - 100 %	45 **	38	fast	49.9	54.7	19.8 – 125.5
			med	32.3	35.4	13 – 80.2
			low	25.0	27.5	10 – 62.7
Fz slope	89 **	71	fast	15.2	20.3	2.7 – 85.5
			med	8.4	11.3	1.5 – 46.9
			low	6.5	8.8	1.2 – 36.6
Fy max	37 **	32	fast	41.6	44.3	18.8 – 91.6
			med	31.1	33.2	14.2 – 68.1
			low	26.3	28.0	12.0 – 57.7
Fy min	41 *	36	fast	25.6	27.7	10.6 – 61.7
			med	28.4	30.8	11.9 - 68.0
			low	20.3	22.0	8.4 – 48.7
Y ratio	64 **	53	fast	1.64	1.94	0.44 – 6.08
			med	1.08	1.28	0.30 – 3.96
			low	1.28	1.51	0.35 – 4.71
Fx max	55 ns	53	fast	22.1	25.3	7.3 – 67.1
			med	15.0	17.1	5.0 – 44.7
			low	10.9	12.5	3.6 – 32.9
Fx min	55 ns	53	fast	21.5	24.6	7.1 – 65.4
			med	13.9	15.9	4.6 – 41.7
			low	10.4	11.9	3.5 – 31.6
X ratio	73 ns	70	fast	1.01	1.25	0.25 – 4.10
			med	1.06	1.32	0.27 – 4.26
			low	1.04	1.29	0.26 – 4.21

CV-1 = coefficient of variation between runs from different birds and sessions, same speed range

CV-2 = coefficient of variation between runs from same bird and session and speed.

Bird or session component of variance statistically significantly different from zero:

** $p < 0.01$ * $p < 0.05$ ns $p > 0.10$

Effect of speed range on the median is statistically significant at $p < 0.01$ in all measurements except the X ratio.

It can be seen from **Table 5.3** that although grouping the data into speed ranges has reduced the coefficients of variation, they are still high, both between birds, and 'within'

birds. The effect of the speed range on the median is significant in all measurements, except x-ratio. Median peak vertical force, propulsion force and vertical loading rate all increase with increasing speed. Median peak braking force is greatest in the medium speed range, however, and so the Y ratio is lowest at medium speeds.

The 95% ranges are still large, even within speed ranges, and the accuracy is not improved much by using extra runs. A greater improvement in the accuracy of the estimate is obtained by increasing the number of runs from 5 to 10, than from 10 to 15.

Table 5.4: Data within speed ranges: means, medians, coefficients of variation and 95% ranges for ground reaction force measurements made on steps.

MEASUREMENT	CV - 1	CV - 2	Speed range	Median	Mean	95 % range for a new observation
Stance time**	22 ns	22	fast	0.250	0.256	0.156 – 0.402
			med	0.312	0.319	0.196 – 0.497
			low	0.396	0.405	0.248 – 0.632
Brake % ns	24 #	22	fast	55.1	55.1	27.3 – 82.9
			med	53.4	53.4	26.1 – 80.8
			low	51.8	51.8	24.3 – 79.3
Braking rate **	51 ns	51	fast	2.18	2.45	0.76 – 6.23
			med	1.78	2.01	0.64 – 5.00
			low	1.00	1.13	0.36 – 2.83
Propulsion rate **	94 ns	90	fast	12.3	16.8	2.2 – 69.0
			med	7.5	10.3	1.4 – 40.9
			low	4.0	5.5	0.7 – 22.3
Braking integral ns	80 ns	74	fast	0.0137	0.0176	0.0030 – 0.0626
			med	0.0173	0.0222	0.0039 – 0.0772
			low	0.0152	0.0194	0.0034 – 0.0681
Propulsion integral ns	84 ns	77	fast	0.0153	0.0200	0.0031 – 0.0758
			med	0.0154	0.0201	0.0031 – 0.0740
			low	0.0177	0.0232	0.0036 – 0.0862

CV-1 = coefficient of variation between runs from different birds and sessions, same speed range
CV-2 = coefficient of variation between runs from same bird and session and speed

Bird or session component of variance statistically significantly different from zero:
# $p < 0.10$ ns $p > 0.10$

Effect of the speed range on the median statistically significant:
** $p < 0.01$ ns $p > 0.10$

Table 5.4 shows that as with the measurements made on runs, the coefficients of variation are reduced when the measurements made on steps are analysed within speed groups. Most coefficients of variation are high, except for stance times and brake % (and therefore prop.%).

The effect of speed on median stance time, braking rate and propulsion rate is significant at $p<0.01$. Median stance time decreases with increasing speed, while both median braking rate and median propulsion rate increase with increasing speed. The effect of speed is not significant on median braking % (and therefore prop.%), or on braking or propulsive integrals. Median brake % (and therefore prop.%) change only slightly with speed ranges, increasing from 51-55% with increasing speed. While the median propulsion integrals show a trend of increasing with decreasing speed, the braking integral is highest in the median speed range.

5. 4 DISCUSSION

In measuring the ground reaction forces produced during walking, it is important to consider the effects of both bodyweight and speed, prior to drawing any conclusions. The effect of bodyweight is obvious; the body exerts a downward force equal to its own mass times gravity. Dividing the resultant forces by bodyweight, and discussing them in terms of ' % bodyweight' allows more useful comparisons to be made between subjects of different weights (Winter, 1985; Budsberg *et al*, 1987; Kadaba *et al*, 1989).

The effects of speed are less easy to control for. The results show that the effect of speed on the median is highly significant ($p<0.01$) in all measurements made on runs, except for X-ratio. Studies on other species have produced conflicting results: some have found positive correlations between speed and peak forces (Barr *et al*, 1995), but others have not (Merkens *et al*, 1988). In the present study, only the X ratio was unaffected by speed, which is not surprising as this is the ratio of peak lateral to medial force, which should be equal in a subject walking in a straight line. With regard to the other forces, in the Brown Leghorn at least, the present study has shown that it is very important to consider the speed at which the bird was moving when defining normal ranges for the ground reaction forces.

It can be seen from the results that the variability in the ground reaction force measurements is very high, not only between different birds in different sessions, but also between the same bird in the same session. As expected from such high coefficients of variation, the 95% ranges for new observations are wide, as are the 95% ranges for newly observed bird means. The accuracy of the estimates is not greatly improved by using an increased numbers of runs, which in itself could increase the variability, as fatigue and

frustration develop. As the effect of speed was significant in all measurements (except X ratio), it is not surprising that dividing the runs into speed ranges and comparing measurements within those speed ranges results in lower coefficients of variation.

Other studies have examined repeatability and sources of variation within gait data. Gait variables were found to be quite repeatable in subjects walking at their natural speed, and the level of repeatability was found to be higher for a subject on the same day, than between days (Kadaba *et al*, 1989). Force plate analysis was suggested to be a highly repeatable measurement technique by Jevens *et al* (1993), who examined the sources of variation in trials using dogs. The coefficients of variation in that study on dogs were considerably lower than those found in the present study on chickens: CV for peak vertical forces and impulses 5.8-8.5%, CV for forelimb cranial and caudal impulses 26.4 and 30.5% and CV for hindlimb cranial and caudal impulses 63 and 25.9% respectively. In general, however, the vertical and craniocaudal forces measured in the present study had lower coefficients of variation (i.e. were more repeatable) than the mediolateral forces, in agreement with work on humans (Kadaba *et al*, 1989). Much of the variability is though to be due to fluctuations in speed as the subjects cross the plate (Jevens *et al*, 1993), and this would certainly be the case with the birds, which take several steps across the plate (as average velocity = stride length x cadence). Even within a step, the instantaneous velocity also varies slightly, being slowest at mid-stance, and fastest during the double contact period (Whittle, 1991). There is also a degree of normal biological variability between limb loading in successive stance phases, even when speed is constant (Merkens, 1987).

Certain studies have grouped force data into stance time periods, rather than speed groups, and this method might have reduced the variability seen in the present experiment. It has to be remembered, however, that disproportionate changes can occur in the stance times of lame animals, and for this reason, using stance times is generally rejected in favour of using speed groups (Riggs *et al*, 1993; McLaughlin and Roush, 1994, 1995; DeCamp, 1997). Another method which has been used is to take a number of runs and average them (after normalising them to stance phase) to produce a 'representative' trace for a subject (Merkens, 1987). This method fails to account for the within subject between run, and within subject between step variability, or any changes in the gait with time. Thus, although it might be useful as a method to produce an 'average' trace for a sound animal, it would be prone to considerable error if used to produce such a trace for a lame animal. Other authors suggest it is acceptable to base decisions on a single trial in normal subjects, but not for subjects with disabilities, whose gait patterns may have lower repeatability (Kadaba *et al*, 1989). This point was clearly illustrated in a human study on active people with a variety of disabilities, which found that in 26% of the trials, the gait was judged to be normal on the first traverse, but abnormal on the second (Gabell and Nayak, 1987). On this basis, it was

decided to present the results of the present study without trying to average them to produce a 'characteristic' pattern.

In subjects that can be persuaded to place only one foot on the plate at a time, the footstep can be clearly identified from the vertical force trace. In this experiment, pilot trials were done using strips of wood placed alternately directly onto the plate, and across the plate, resting on blocks either side (and so not contacting the plate). When the bird stepped on one which rested on the plate, the force would register, when it then stepped onto one placed across the plate, the trace would drop back to zero. This method was very inefficient, however, producing useful results on average in 1 in 10 trials. Instead, as described in the introduction, individual steps were identified for analysis using the craniocaudal force trace, to allow comparison of the forces over a standardised period of one stance phase.

Examination of GRF graphs clearly shows that the braking force starts almost simultaneously with the rise in the vertical force (as the foot is placed on the ground), and the propulsion force drops to zero almost simultaneously with the vertical force, as the foot is lifted from the ground (Leach, 1987; Rumph *et al*, 1994; Keg *et al*, 1996). Although there is always a double contact period in walking, work on both quail (Clark and Alexander, 1975) and horses (Merkens *et al*, 1986) has shown that the Fy curves of the consecutive braking and propulsive limbs followed each other with only a small overlap, so that effectively only one limb contributed at a time. The braking can only come from the foot being placed down, as the propelling only comes from the foot that is about to be lifted, and the forceplate registers the net result of the two forces.

The fact that the vertical force trace never returns to zero when the bird is crossing the plate means that only the initial vertical loading rate can be measured as the bird steps onto the plate. Vertical integrals are likewise unavailable for individual steps. It also made it impossible to determine whether the vertical force traces of birds showed the two maxima and single minimum seen in a characteristic human footstep. In their study of quail, however, Clark and Alexander (1975) comment that the records of vertical force generally showed twin peaks.

MEASUREMENTS MADE OVER WHOLE RUNS:

Vertical forces

The shape of the vertical force trace results from the force involved in supporting the bodyweight, plus the force required to produce vertical acceleration of the CG during walking (Collins and Whittle, 1989). As discussed in the introduction, force is required to accelerate the CG upwards in early stance (moving in an arc 'over' the hip), the force then decreases as the knee flexes slightly in mid-stance, then increases again as the leg extends again. As the CG passes the 'noon' position above the stance leg, it begins to fall downwards and forwards under gravity, thus the vertical force decreases, before increasing again as the other leg starts to provide support and reaccelerate the CG upwards again (Whittle, 1991).

In the present study, median peak vertical force averaged 132.5% of bodyweight, which is of a similar order of magnitude to the vertical forces seen in human walking: 125-149.9% of bodyweight (Betts *et al*, 1980a; Whittle, 1991). These values are similar to the estimates of net vertical force made for the Brown Leghorns in the pedobarograph work (Chapter 3). Interestingly, they are also similar to the peak value of 127% of bodyweight reported for vertical force in quail running at a mean velocity of 0.5m/sec. (Clark and Alexander, 1975).

The peak vertical forces in the present study increased significantly with increasing speed ($p<0.01$), which is in agreement with work on other species (Riggs *et al*, 1993; McLaughlin and Roush, 1994). In a standing subject, the average vertical GRF must equal the bodyweight (mass x gravity). If the subject is moving, the stance period of each foot is shorter, and so the force has to be greater e.g. if in contact for half the time, the force will have to be 2(mass x gravity) (Alexander, 1977). It can also be explained in terms of change of momentum (force = rate of change of momentum); with increasing speed, the foot is placed down more quickly, the CG is accelerated up and over the stance leg and then drops, to be accelerated upwards again with the next step. The faster this occurs, the greater the rate of change of momentum with each step, and therefore the greater the force required.

The peak vertical forces were found to be much more normally distributed if the 'normalised' peak had 100 % of the bodyweight subtracted i.e. considered as % above bodyweight. This transformation can be applied to data collected over a run as the vertical force will always equal or exceed the bird's bodyweight as it crosses the plate. A similar transformation may not be suitable for data collected from an individual step however, as the bird may not take 100% of its bodyweight onto one limb during a step, especially if the limb is painful. It is difficult to explain why the data is more normally distributed when the

bodyweight is removed. A possible explanation is that during walking, as discussed above, a subject basically raises their CG by straightening their leg, and then falls forward under gravity. On 'hitting' the ground, the kinetic energy (Ek) = force x vertical distance (d), which can be re-arranged as force = mv*v / (2*d), where m = mass, and v = velocity. Assuming that leg length increases proportionally with mass, then the larger birds will produce greater downward forces in walking due to a combination of their greater weight and higher velocity. Subtracting the bodyweight may partly compensate for the differences, and therefore make the distribution more normal.

Two other aspects of the vertical force are worth mentioning. In vertical force traces of human walking, there is often an initial heelstrike impulse, however this was not apparent for the birds. This is a very high frequency impulse, however, and can be 'lost' if either the sampling rate, or the resonant frequency of the plate, is too low (Collins and Whittle, 1989). These impulses would not occur, however, if the birds decelerate their feet just before ground contact, so that they are placed down more gently (Collins and Whittle, 1989).

The rate at which the bodyweight was loaded onto the foot as it first contacts the plate (Fz slope) was also extremely variable even within speed ranges. The loading rate increased with speed however, almost doubling from the medium to the fast speed range. This is not surprising, again if we consider that vertical force increases with speed, and as stance time decreases with speed, the force would have to be more rapidly loaded onto the leg over a shorter period.

The peak vertical forces and impulses are generally the least variable GRF's, and have been suggested by some authors to be the most useful measures of normal and abnormal gait (Budsberg *et al*, 1987; Jevens *et al*, 1993, Rumph *et al*, 1994). The results of the present study have shown that the median vertical forces are significantly affected by speed, however, and so it is important to evaluate results within the appropriate speed range.

Craniocaudal force

In this study, peak propulsive forces were in the range of 26.3 - 41.6% of bodyweight, and increased significantly with increasing speed. Peak braking forces were considerably lower than the peak propulsive forces in the same speed range, and were not significantly affected by speed. In general, to maintain a steady speed across the plate (one of the criteria on which the runs were selected), the braking and propulsive forces should be equal. This would result in a smooth forward progression of the CG, and would be reflected in an Y ratio of 1 (as was seen in the medium speed range). When the forces are measured over a run, however, the individual steps within the run can vary. In the runs where the peak

propulsive forces were greater than the peak braking forces, the birds may have actually been accelerating across the plate. However the same result could arise if one of the steps had an unusually large propulsive force, while the others had much lower propulsive forces, and slightly higher braking forces[1].

In order to make comments about speed, therefore, the average forces over the run might be more useful, or the measurement over an individual step.

It is not surprising that the peak propulsive forces increased significantly with speed, while the braking forces did not. As force equals rate of change of momentum, to increase forward speed, while keeping direction and mass the same, the propulsive force must be increased. In humans, for example, the greatest rate at which energy is generated across any joint occurs during the push-off phase, when ankle plantar flexion generates up to 500W of power (Whittle, 1991). The braking force arises as friction stops the foot sliding forwards as the weight starts to be loaded onto the limb (which is still at an angle with the ground), and so would not tend to increase to the same extent with increasing speed. It was however surprising that the peak braking force was highest in runs in the 'medium' speed range. At slow speeds, the foot may be decelerated prior to being placed down, and as the speed is slower, it is likely the steps will be shorter, and the stance phases longer – thus the friction forces will be lower, and the rate of change of momentum less. At fast speeds, the reverse will apply. The higher braking forces at medium speeds are more difficult to explain however. The forward deceleration of the CG is greatest when the line from the CG to the foot is more steeply inclined (Cavagna and Margaria, 1966). With increasing speed, step length increases, implying a greater deceleration with each step. It is possible that the medium speed birds had longer step lengths than the fast birds, whose greater speed may have been produced by a greater number of slightly shorter steps. This hypothesis is supported by the results from the Brown Leghorns in Chapter 3, which suggested that the birds increased speed more by increasing step frequency than step length.

It is interesting that the peak propulsive force is of similar magnitude to the peak vertical force in the same speed range, if the bird's bodyweight (i.e. 100%) is subtracted. This is quite an inefficient way of walking, if as much force is being put into raising the CG vertically as moving it forwards during locomotion. Although the vertical excursions of the CG initially increase as step length increases in human walking (Cavagna and Margaria,

[1] It is interesting to speculate on the motivation of the birds being reflected in their preference to accelerate or decelerate when crossing the plate. It is possible that the more nervous birds are moving at higher speeds, and accelerating away from the perceived threat of the handler. The slower moving birds could either be less motivated to 'escape', or simply less able to move faster. It is possible that the birds that moved at the medium speeds, with a Y ratio closer to one, were more comfortable in the testing environment.

1966), the various gait optimisations prevent this becoming too extreme (Whittle, 1991). Either birds are unable to make use of similar optimisations, as discussed in Chapter 4, or their gait pattern changes, for example, their legs bend more and they have a more 'bouncy' gait at increasing speeds.

Unfortunately, Clarke and Alexander (1975) do not quote figures for craniocaudal forces in their study of quail, but simply comment that they were 'substantial', and larger (relative to the weight of the animal) than in human walking. The results of studies on other species are variable: some have shown a significant positive correlation between peak craniocaudal forces and increasing velocity in dogs, for example (McLaughlin and Roush, 1994), while others have not (Riggs *et al*, 1993). Overall, the craniocaudal forces in the present study had the lowest coefficients of variation of all the measurements made on the runs, suggesting this was one of the less variable aspects of the gait pattern in birds.

Mediolateral force

The mediolateral forces move the CG from side to side during walking, to position it over the stance leg. The greater the walking base, the greater the excursion required and the more inefficient the gait (Whittle, 1991). A combination of narrowed walking base (e.g. positioning the feet more under the body), and gait optimisations such as lateral trunk bending, effectively minimise the lateral excursions in most species. They have been variably quoted as <5- 8% bodyweight in humans (Biewener, 1992), 'small' in quail (Clark and Alexander, 1975), and between 1.28-6% bodyweight in dogs (Budsberg *et al*, 1987; Riggs *et al*, 1993). Due to the small size (and variable nature) of the mediolateral forces, they are usually ignored in gait studies (Leach, 1987; Keg *et al*, 1996). As discussed in Chapter 4 however, chickens do not minimise their lateral movement very effectively, and so it is not surprising to find peak mediolateral forces of 10.4 - 22.1% of bodyweight being produced during walking in this study. Although the coefficients of variation were high, the forces were of sufficiently large magnitude to suggest that this is one way in which birds differ dramatically from most other quadrupedal and bipedal species.

In human walking, most of the time one foot is on the ground, it is accelerating the CG towards the opposite side of the body (Whittle, 1991). It is more difficult to measure mediolateral forces in quadrupeds, however, and they appear to be more variable. Work on dogs suggests that the usual mediolateral force pattern is laterally directed in the forelimbs, but variable in the hind, with significant differences in both mediolateral force and torque between the sides (Rumph *et al*, 1994). In theory, if the gait optimisations are effective, speed should have little effect on mediolateral forces. This was the case in the study in dogs,

which found no significant change in peak mediolateral force with speed (Riggs *et al*, 1993, who quotes similar findings in various human studies). In the present study, however, the mediolateral forces increased significantly with increasing speed in the chicken; the lateral forces were approximately twice as great in the 'fast' compared to the 'slow' speed ranges. The same principle therefore applies as to the other forces – force being rate of change of momentum, the faster moving bird has to push its CG over in the opposite direction more rapidly during each step. In subjects with effective optimisations, however, the increase in speed is unlikely to have a significant effect. Speed had no significant effect on the X ratio however, (lateral force / medial force), which is approximately equal in all ranges. This would be expected, as the 'side-to-side' movement in bipeds should be equally balanced over a series of steps, or the run would not be straight. This would not be the case with an individual step, however, as the greatest force is the one pushing the CG over the leg beginning its stance phase. (The subsequent step should produce the same magnitude of forces in the opposite directions). This suggests that the X ratio (over two subsequent steps) could be a useful indicator of lameness, as it is unlikely to be one in birds with a unilateral lameness, when the bird is 'protecting' one leg. Despite the fact that many studies ignore the mediolateral forces, some workers have suggested that they may in fact be one of the better GRF's to use as an indicator of gait abnormalities (Andriacchi *et al*, 1977; Leach *et al*, 1987). Other studies, such as that by Merkens and Schamhardt (1988b) using experimentally-induced lameness in horses, found only small changes in the mediolateral forces.

MEASUREMENTS MADE ON AN INDIVIDUAL STEP FROM A RUN:

Stance times

Stance time decreased significantly with increasing speed ($p<0.01$), as would be expected. This is in agreement with studies on humans (Mann and Hagy, 1980; Whittle, 1991), and dogs (McLaughlin and Roush, 1994). Within each speed range the coefficients of variation for the stance times were quite low, compared to the other parameters.

Figure 5.2 shows simplified craniocaudal force curves (from birds) over a single stance period, in the three speed ranges. This illustrates some of the points to be made in the last part of the discussion.

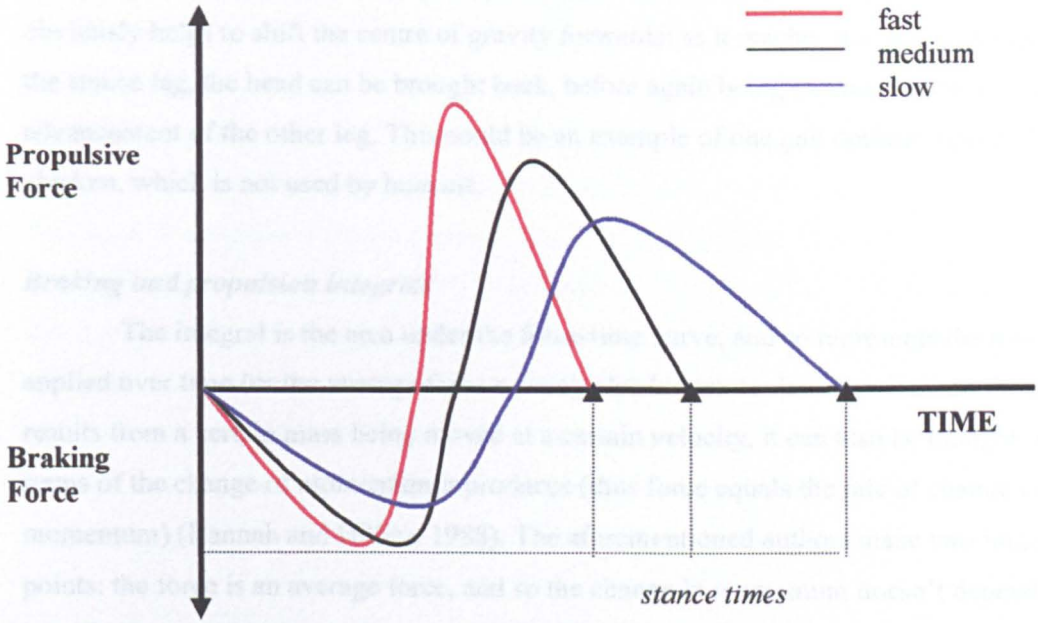


Figure 5.2: Simplified curves for craniocaudal ground reaction forces in each speed range

Similar basic principles apply when the effect of speed on the GRF's is assessed over an individual step, rather than a run. Increasing speed results in a decrease in stance time, and so the same force needed to support and propel the body forwards would have to be applied at a greater rate.

Braking and propulsion rates

As predicted, both braking and propulsion rates increased significantly with increasing speed ($p<0.01$). Both of these parameters were highly variable between individual steps even in the same speed range, the CV for the propulsion rate being as high as 90%. The median propulsion rate was approximately 4 times as high as the braking rate at slow and medium speeds, and up to 6 times as high at fast speeds. This demonstrates the greater rate at which the 'push-off' thrust is applied at faster speeds. While the main propulsive thrust in humans comes from rapid plantar flexion of the ankle (Whittle, 1991), the GRF's are also affected by the action of other body segments (Winter, 1985). It has been shown, for example, that the swing leg also has a major influence on propulsion during human walking (Dillingham *et al*, 1992), as does the action of the trunk twisting about the vertical axis, and the arms swinging out of phase with the legs (Whittle, 1991). Birds appear not to be able to twist their trunks much, and obviously do not swing their wings, but it is interesting to consider the effects of their head movements. Analysis of the videotapes

shows that the birds extend their heads forwards as they are swinging the leg forwards, then bring their heads back as the foot contacts the ground and weight is loaded onto it. This obviously helps to shift the centre of gravity forwards; as it reaches the 'noon' position over the stance leg, the head can be brought back, before again being extended forward with advancement of the other leg. This could be an example of one gait optimisation in the chicken, which is not used by humans.

Braking and propulsion integrals

The integral is the area under the force-time curve, and so represents the total force applied over time (or the average force x time), also known as the 'impulse'. As the impulse results from a certain mass being moved at a certain velocity, it can also be thought of in terms of the change of momentum it produces (thus force equals the rate of change of momentum) (Hannah and Hillier, 1988). The aforementioned authors make two important points: the force is an average force, and so the change in momentum doesn't depend on how the force varies during the time; and the momentum can change due to a change in speed, or vector change in velocity, or a change in mass. In theory, therefore, as speed increases, the speed of the foot increases, the mass stays the same, so the velocity should increase, and so therefore should the integral. In the present study, however, neither braking nor propulsion integrals changed significantly with speed (although the trend was for both to increase with decreasing speed, as stance time increased). As the peak forces increased (as did the rate at which they were applied), the time during which the force was applied must have decreased if the total force remained the same. This is in fact what happened, as reflected in the significant decrease in stance time with speed.

Studies on quadrupeds have reported variable effects of speed on braking and propulsion integrals. A study on dogs found significant decreases in vertical, braking and propulsion integrals as stance time decreased (McLaughlin and Roush, 1994). Other workers have failed to find significant trends, however, despite claiming that braking impulses consistently increased with increasing velocity (Riggs *et al*, 1993). It is possible that no statistically significant trends were found in the present study due to the relatively small range of speeds demonstrated by the birds in comparison to other species.

% stance time spent braking or in propulsion

The % stance time spent in braking or propulsion was approximately 50-50, and did not change significantly with speed. This is in agreement with the results of other studies (McLaughlin and Roush, 1995), which have suggested that the change in speed occurs mainly by a change in the rate of application of the propulsive force (while the duration stays the same) (Wetzel and Stuart, 1977). This was demonstrated in the present study,

where the overall decrease in stance time with increasing speed was accompanied by increases in both braking and propulsion rates.

Summary:

With increasing speed: overall stance time decreases, but the % of stance time spent braking or in propulsion remains approximately equal. The peak propulsive forces increase, as does the rate at which these forces are generated. The peak braking forces are variable, being greatest at medium speeds, although the rate at which the forces are generated increases. Despite the overall increase in peak propulsive force, this is produced at a greater rate, over a shorter time, so the propulsive integral decreases. The braking integral is variable, being greatest in the medium speed range, as the higher peak forces are developed at a similar rate, but over a longer stance period, than at fast speeds.

This study has quantified the three ground reaction forces in Brown Leghorn hens, and found them all to be very variable, even when speed has been controlled to an extent. The significant effect of speed on the ground reaction forces demonstrates that it is important to consider the speed at which the bird was moving before deciding whether a particular value is abnormal. While the vertical and craniocaudal forces showed similar characteristics to those of other species, the mediolateral forces were found to be 2-3 times greater in these birds.

CHAPTER 6:

A FORCE PLATE STUDY OF THE GAIT OF BROILERS (RAISED ON DIFFERENT FEEDING REGIMES, AND SUBSEQUENTLY TESTED WITH ANALGESIC).

6. 1 INTRODUCTION

This experiment was designed to study the ground reaction force patterns (produced during walking) by groups of broilers raised on different feeding regimes. As in Chapter 4, comparisons were made between birds at the same age, and at the same bodyweight, in order to test the hypothesis that GRF patterns can be influenced by growth rate.

In the second part of the experiment, the possibility that the gait of broilers is influenced by pain is investigated. The birds were given a course of analgesic (carprofen), or a placebo, and the gait re-analysed. Changes in the gait pattern of birds given analgesic would support the hypothesis that the original locomotor pattern of the bird was affected by pain.

6. 2 MATERIALS AND METHODS

Equipment

The Kistler force plate is described in detail in Chapter 2.2. and the set-up of the runway and equipment is described in Chapter 5.2.

The testing set-up is shown in Figure 2.6.

In this experiment the sampling rate of the force plate was set at 100Hz, over a period of 6 seconds, as the broilers walked more slowly across the plate than the Brown Leghorns.

Birds (see Chapter 2.4)

Forty male Ross 308 chicks were obtained as day-olds, and fed *ad libitum* for the first 11 days, after which they were randomly separated into two groups. One group continued to be fed *ad libitum*, the other was fed according to the Ross PSM restriction ration (Appendix 2.2). The groups were housed in separate floor pens (1.75m x 1m), on deep litter wood shavings. (This resulted in a stocking density of approximately 11 birds /m^2 when fully grown, which is at the low end of the standard commercial densities of 9-28 birds / m^2). The pens were built at one end of the runway, and were separated by a solid wall to prevent the birds from seeing each other. In this experiment, the pens were enclosed by mesh, so that while the birds could see out, they couldn't escape to roam. The birds were fed on Roslin starter diet throughout life (Appendix 4.1), and *ad libitum* water was available to both groups.

Initially, extra heating was provided in each pen using a standard broiler lamp (with a 250W bulb), until day 11. The room temperature was set at 20^{o}C, and the humidity

maintained at around 50%. Room lighting was provided by 10 lamps, each with 60W bulbs installed; the 'daylength' was reduced from 23 hours on day 1 to 14 hours by day 5, thereafter being maintained on the 14 hours light: 10 hours dark regime. As before, and additional fluorescent strip light was positioned over the runway, to provide extra illumination for the video cameras.

Drugs

Half of the birds in each food group were randomly allocated one of two drug treatments:

a) Carprofen (Zenecarp solution, 50mg/ml): an aqueous solution containing carprofen 5%w/v with Ethanol Ph Eur 10% v/v as preservative. Carprofen is a non-steroidal anti-inflammatory drug, with analgesic and anti-pyretic properties. It is routinely used in the treatment of musculoskeletal disorders in a variety of species, and for the control of post-operative pain. It was administered at the recommended dose rate of 4mg/kg subcutaneously (S/C) (Veterinary data sheet compendium, 1998-1999). Although the efficacy of carprofen has not been proven in birds, anecdotal evidence suggests that it is an effective analgesic in birds (J.Cooper, BSAVA lecture, 1998). This is supported by a recent study which demonstrated that lame broilers 'self-administered' the drug by choosing to eat a greater quantity of food containing carprofen (Pickup *et al*, 1997).

b) Placebo (carprofen placebo, batch JB151, supplied by Grampian Pharmaceuticals): identical to the Zenecarp solution, minus the active ingredient (carprofen 5%). It was administered at a dose rate of 0.08ml/kg S/C, so that the same quantity of solution was injected for the equivalent dose of carprofen.

Each drug was administered as a once daily S/C injection (into the 'scruff' of the neck), for 5 consecutive days, with care being taken to avoid the dorsal air sacs.

Experimental Design and Testing

Of the original 40 chicks, 3 from the restricted-fed group and 4 from the *ad libitum*-fed group were culled for general weakness, poor growth or respiratory problems (although there were no significant findings on subsequent *post-mortem* examination). The remaining birds grew rapidly, and so were heavier than expected when testing began at 6 weeks. As in previous experiments, minimal training was undertaken, the birds being familiarised with the runway and testing procedure in the week before the experiment began.

The experimental design was as follows:

Day 1: half of the birds in each food group were tested in random order on the forceplate,

until an average of 10 runs was obtained from each bird. The *ad libitum*-fed birds that had been tested were then randomly allocated into one of two groups: the birds in one group were given a dose of carprofen (4mg/kg S/C), and those in the other group received a similar quantity of placebo (0.08mls/kg).

Day 2: the same protocol was repeated with the other half of the birds from each group. The appropriate injection was administered to the *ad libitum*-fed birds once daily for 5 days. At the end of the treatment course, 10 more runs were collected from each *ad libitum*-fed bird, as described previously. The *ad libitum*-fed birds hock joints were examined and scored, after which the *ad libitum*-fed birds were killed by an overdose of anaesthetic (Pentobarbitone sodium, Merial Animal Health), and samples taken for *post-mortem* examination.

The restricted-fed birds were grown to approximately the same bodyweight as the *ad libitum*-fed birds had been when they were first tested at 6 weeks. A similar treatment regime was then applied to the restricted-fed birds - half the birds were tested on one day, and then given either carprofen or placebo; the following day the remainder were tested, followed by administration of treatment. After 5 days on either carprofen or placebo, the birds were re-tested, 10 more runs collected from each. The birds hock joints were examined and scored, after which the birds were killed by an overdose of anaesthetic (Pentobarbitone sodium, Merial Animal Health), and samples taken for *post-mortem* examination.

Data was therefore obtained for:

- *ad libitum*-fed and restricted-fed birds at 6 weeks of age
- *ad libitum*-fed and restricted-fed birds at the same average bodyweight
- *ad libitum*-fed birds before and after either carprofen or placebo
- restricted-fed birds before and after either carprofen or placebo

Gait Parameters *(see Chapter 2.3)*

The following gait parameters were measured: speed, cadence, peak ground reaction forces (vertical, craniocaudal and mediolateral), vertical loading rate, braking and propulsion rates and braking and propulsion integrals. Speed and cadence were calculated from measurements made from the video films, the other parameters were measured using the force plate. The same abbreviations are used in the tables as in Chapter 5.

Hock scoring and post-mortem analysis:

Immediately prior to euthanasia, the birds hock joints were examined for signs of inflammation, and graded for heat, swelling and bruising on a simple objective scale, where:

0 = absent

1 = slight

2 = moderate

3 = marked

The range of movement (rom) of the joint was also assessed (as normal, increased or decreased) as was the presence or absence of crepitus.

After the birds were killed, synovial fluid samples were taken from the hocks for analysis. The fluid was collected using a 21G x 1" needle, attached to a 5 ml syringe, inserted into the joint from the caudolateral aspect. The synovial fluid samples were then 'scored' on various characteristics: volume, turbidity, colour, viscosity and presence of fresh or old blood. The SG was measured using a refractometer (Houlton, 1994), on the urine specific gravity scale of 1.000-1.050 (Atago Urine Specific Gravity refractometer, Atago, Japan). The pH was measured using pH indicator paper (pH 4.5-10, Whatman).

A smear was made from each fluid sample, air dried and stained with May-Grunwald-Giemsa (see Appendix 7.1). A sample of synovial membrane was then collected from the anterior aspect of each hock joint, fixed in 10% buffered neutral formalin, and processed as described in Appendix 7.2. The results of the subsequent cytological and histological examination of these samples are presented and discussed in Chapter 7.

The legs were removed at hip joint, the soft tissue removed, and the bones stored at - 20°C for later analysis. At a subsequent stage, each tibiotarsus was analysed as described in Chapter 4, for evidence of tibial dyschondroplasia, tibiotarsal rotation or an abnormal tibial plateau angle.

Data Analysis:

As before, the forces were 'normalised' and expressed as percentages of the birds bodyweight, and certain measurements made on steps were expressed as percentages of the total stance time. Measurements that were not normally distributed were transformed to logarithms prior to analysis, and the results are presented as medians. Standard errors of the medians (#SE median) were calculated from first approximations (Kendall and Stuart, 1963). Three levels of factor were considered in the analysis: time, food group and injection, and as the data set was unbalanced, the standard errors of the differences were different. The maximum SE of the differences for the same level of factor was used in the

analysis to produce conservative estimates of probability using *t*-tests (for 28 degrees of freedom).

6. 3 RESULTS

In order to make the analysis as relevant and valid as possible, the feed groups were divided (analytically) at 6 weeks into those birds which subsequently received carprofen, and those which subsequently received placebo. In the series of graphs, the following abbreviations are therefore used in the legends:

A-c: *Ad libitum*-fed group that received carprofen

A-p: *Ad libitum*-fed group that received placebo

R-c: Restricted-fed group that received carprofen

R-p: Restricted-fed group that received placebo

Thus at the 6 week stage, for instance, 'A-c' refers to the *ad libitum*-fed birds which subsequently received carprofen. On the horizontal axis, the 'post-treatment' tick marks refer to the results obtained after 5 days of treatment (the first being the *ad libitum*-fed birds, the second being the restricted-fed birds).

MEASUREMENTS MADE ON RUNS

There were no significant differences in the gait parameters (measured over runs) in any of the birds following administration of carprofen. There was a significant increase in speed of the *ad libitum*-fed birds following placebo administration ($p<0.01$), but no significant changes in any of the other parameters. There were no significant differences within each feeding group between the birds given carprofen or placebo.

Several significant differences were apparent between the two feeding groups however, when the birds were compared at the same ages, or same bodyweights.

Figure 6.1: Bodyweight

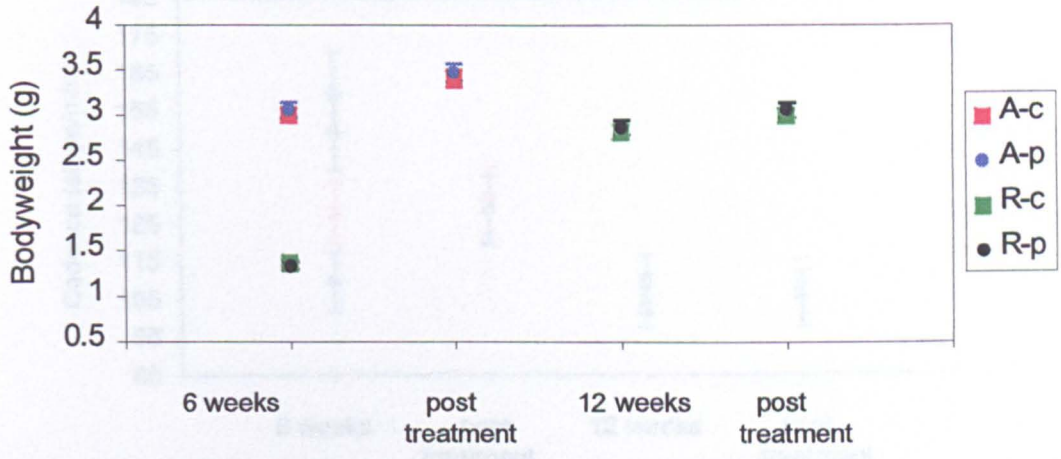


Figure 6.1: median bodyweight (±SE median). Bodyweight was significantly greater in the *ad libitum*-fed birds than the restricted-fed birds at 6 weeks ($p<0.001$), and also increased significantly in both groups during their course of injections: *ad libitum*-fed birds ($p<0.001$), and restricted-fed birds ($p<0.05$). Thus although there was no significant difference in weight between the *ad libitum*-fed or restricted-fed birds at the start of the treatments, the *ad libitum*-fed birds were significantly heavier than the restricted-fed birds by the end of the 5 day treatment course ($p<0.01$-0.001).

Figure 6.2: Speed

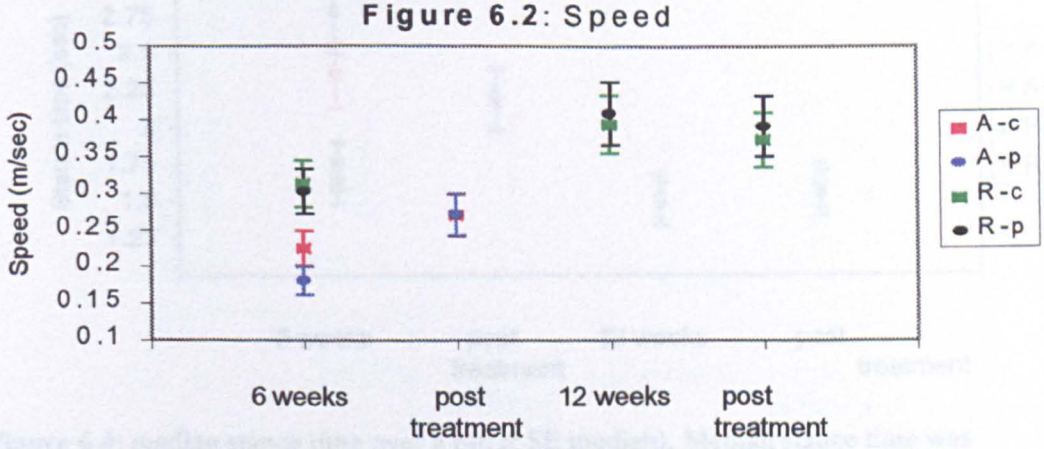


Figure 6.2: median speed (±SE median). Median speed was significantly greater in the restricted-fed birds than the *ad libitum*-fed birds at the same age (6 weeks, $p<0.05$), and at the same bodyweight ($p<0.01$). The median speed of the pre-carprofen restricted-fed birds was not significantly different at 6 weeks and 12 weeks, but that of the pre-placebo restricted-fed birds was significantly greater at 12 weeks ($p<0.05$).

Figure 6.3: Cadence

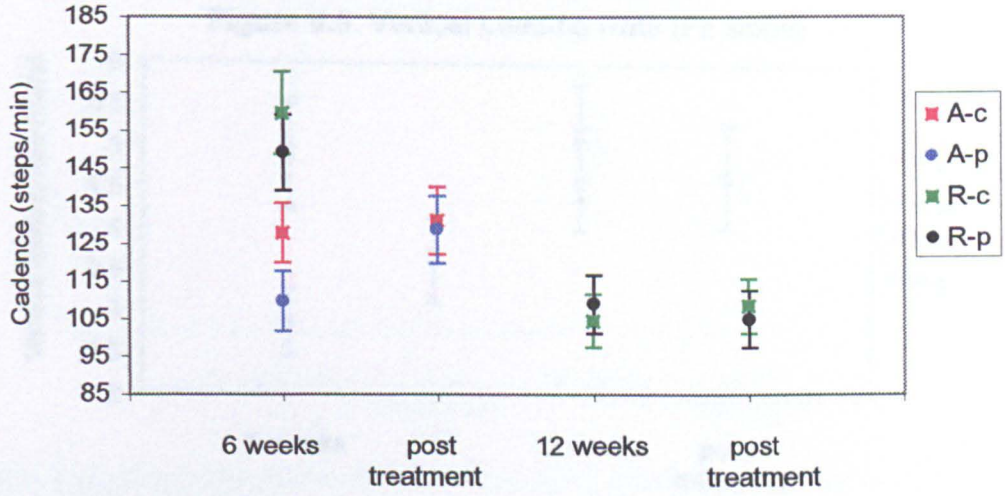


Figure 6.3: median cadence (±SE median). Median cadence was significantly greater in the restricted-fed birds than the *ad libitum*-fed birds at 6 weeks ($p<0.05$-0.01), however there was no significant difference when both groups were at the same bodyweight. Median cadence was significantly greater in the restricted-fed birds at 6 weeks compared to 12 weeks ($P<0.05$-0.01).

Figure 6.4: Stance time over a run

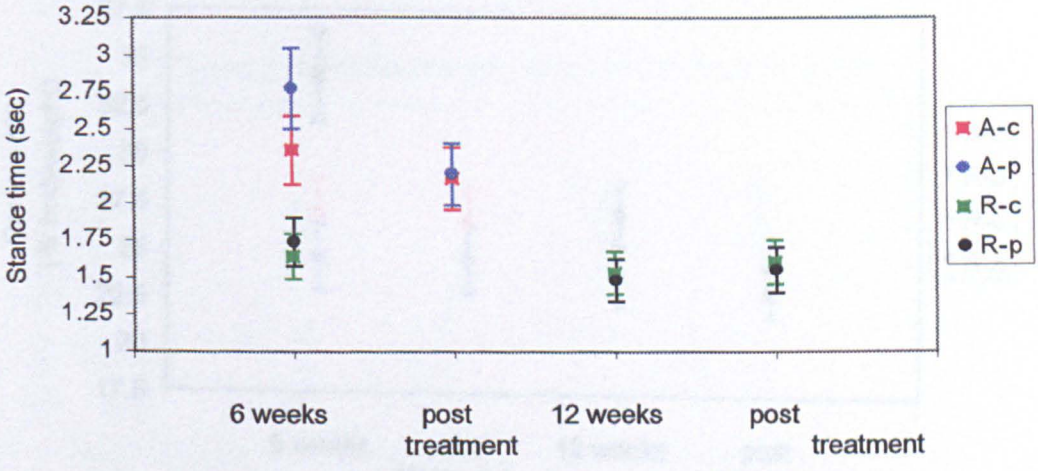


Figure 6.4: median stance time over a run (± SE median). Median stance time was significantly greater in the *ad libitum*-fed birds than the restricted-fed birds at the same age (6 weeks, $p<0.05$-0.01), and at the same bodyweight ($p<0.01$-0.001). There was no significant difference in median stance time in the restricted-fed birds at 6 weeks compared to 12 weeks.

Figure 6.5: Vertical Loading Rate (Fz slope)

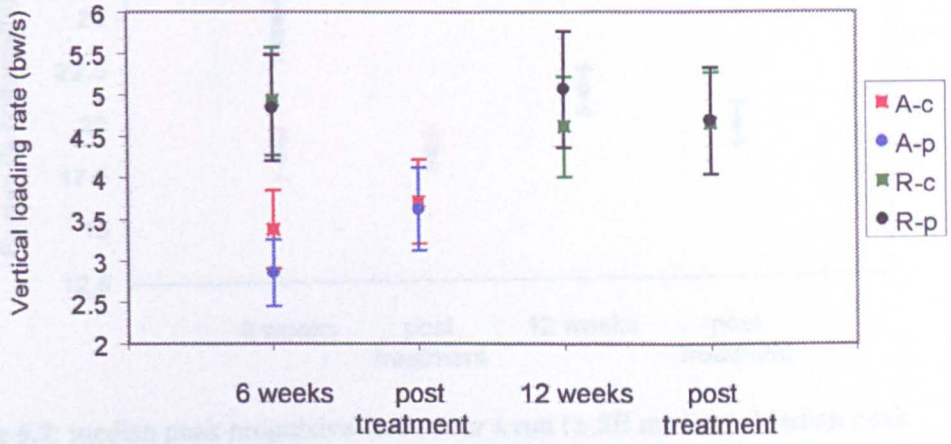


Figure 6.5: median vertical loading rate (± SE median). Median vertical loading rate was significantly lower in the *ad libitum*-fed birds given placebo than the restricted-fed birds given placebo at the same age, and the same bodyweight ($p<0.05$). There were no significant differences between the carprofen groups.

Figure 6.6: Peak vertical force (Fz max-100) over a run

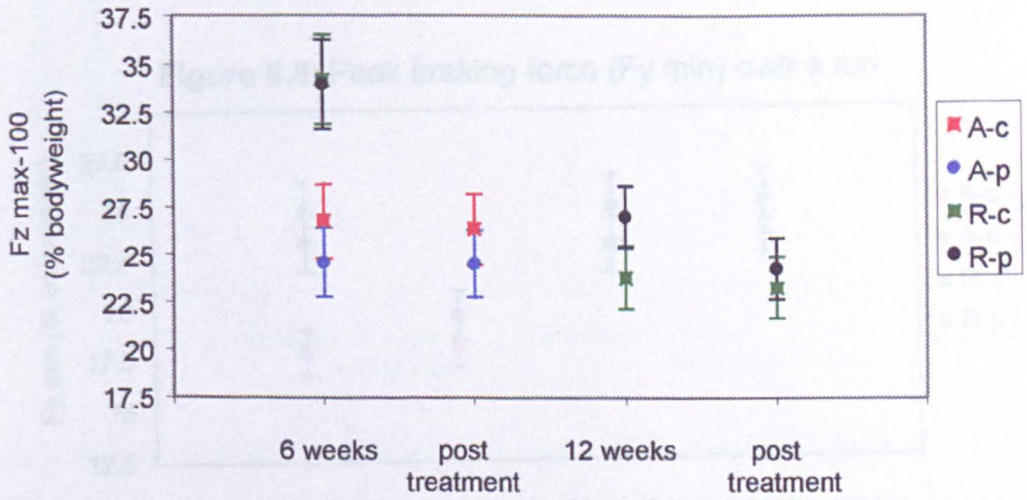


Figure 6.6: median peak vertical force over a run (± SE median). Median Fz max-100 was significantly greater in the restricted-fed birds than the *ad libitum*-fed birds at the same age (6 weeks, $p<0.05$-0.01), but there was no significant difference at the same bodyweight. Median Fz max-100 was significantly higher in the restricted-fed birds at 6 weeks compared to 12 weeks ($P<0.01$-0.001).

Figure 6.7: Peak propulsive force (Fy max) over a run

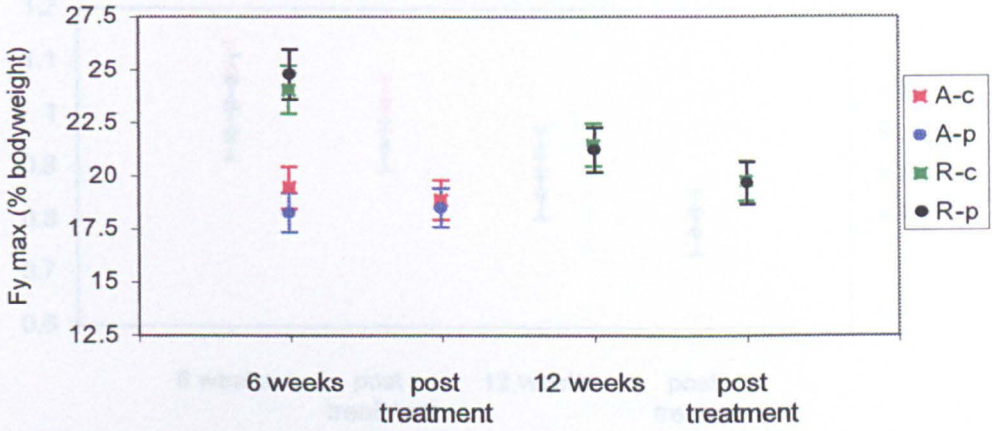


Figure 6.7: median peak propulsive force over a run (± SE median). Median peak propulsive force was significantly greater in the restricted-fed birds than the *ad libitum*-fed birds at the same age (6 weeks, $p<0.001$). At the same bodyweight, the differences were less clear: while median peak propulsive force was significantly higher ($p<0.05$) in the restricted-fed birds (pre-placebo group) than the *ad libitum*-fed birds (pre-placebo group), there was no significant difference between the restricted and *ad libitum*-fed pre-carprofen groups. Median peak propulsive force was significantly higher in the restricted-fed birds at 6 weeks compared to 12 weeks ($P<0.05$-0.01).

Figure 6.8: Peak braking force (Fy min) over a run

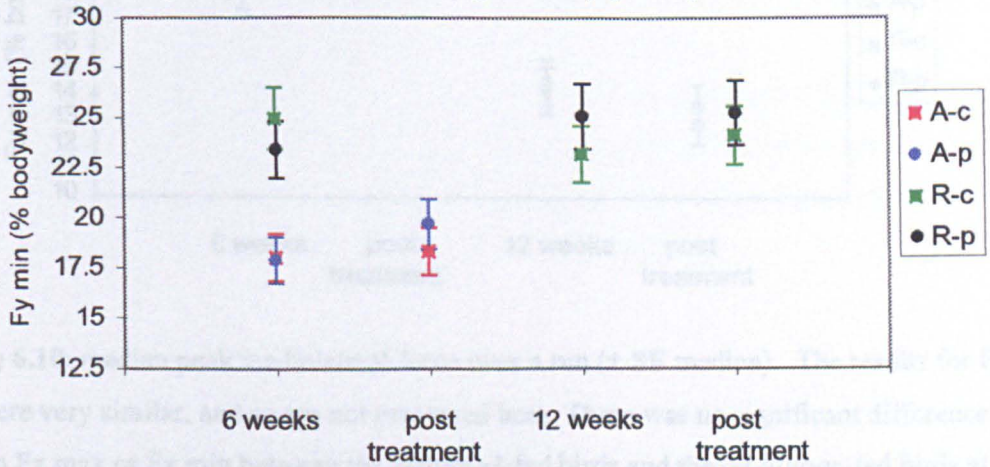


Figure 6.8: median peak braking force over a run (± SE median). Median peak braking force was significantly greater in the restricted than the *ad libitum*-fed birds at the same age (6 weeks, $p<0.01$), and at the same bodyweight ($p< 0.01$-0.001). There was no significant difference in peak braking force between the restricted-fed birds at 6 and 12 weeks

Figure 6.9: Y Ratio over a run

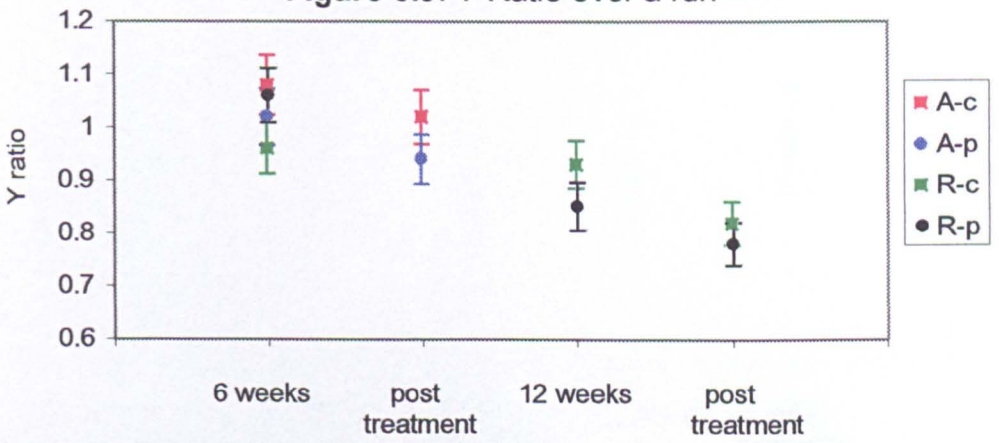


Figure 6.9: median Y ratio over a run (±SE median). Median Y ratio was significantly different only in the pre-placebo restricted-fed birds, being significantly greater at 6 than 12 weeks ($p<0.01$). Where the Y ratio is less than one, this indicates that the peak braking forces were greater than the peak propulsive forces.

Figure 6.10: Peak mediolateral force (Fx max) over a run

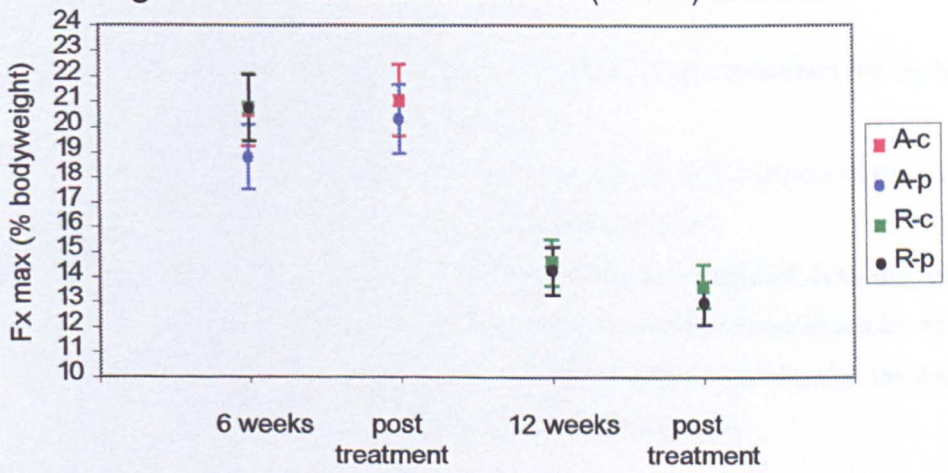


Figure 6.10: median peak mediolateral force over a run (± SE median). The results for Fx min were very similar, and so are not presented here. There was no significant difference in median Fx max or Fx min between the restricted-fed birds and the *ad libitum*-fed birds at the same age (6 weeks), but median Fx max and Fx min were significantly higher in the *ad libitum*-fed birds than the restricted-fed birds at the same bodyweight ($p< 0.05$-0.01). Median Fx max and Fx min were significantly greater in the restricted-fed birds at 6 weeks compared to 12 weeks ($p<0.001$).

Figure 6.11: X Ratio over a run

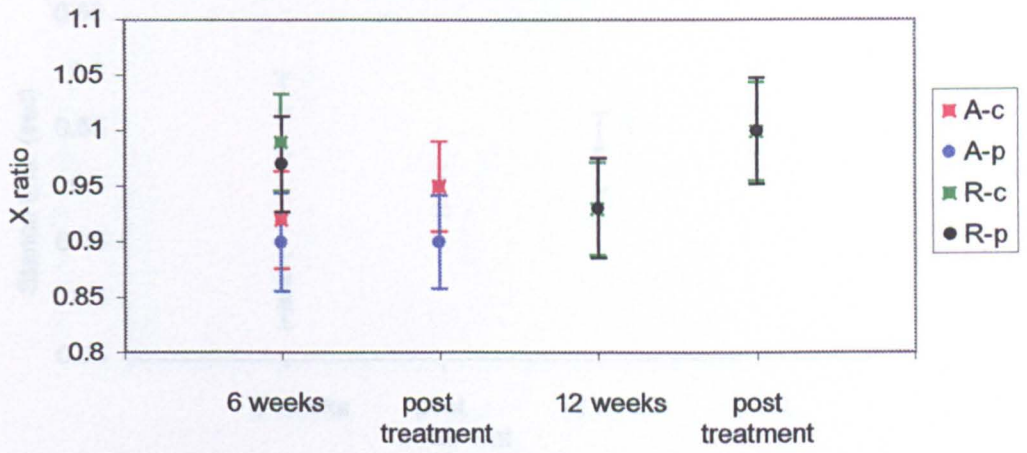


Figure 6.11: median X ratio over a run (± SE median). There was no significant difference in x ratio between any of the groups or treatments at any time.

MEASUREMENTS MADE ON INDIVIDUAL STEPS

Statistically significant treatment effects were found in:

- median braking integral, which was significantly greater in the restricted-fed birds following administration of carprofen ($p<0.05$)
- mean % stance time spent in propulsion, which was significantly greater in the restricted-fed birds following administration of carprofen($p<0.05$)

There was no significant difference in any of the parameters measured, between the birds within a group receiving either carprofen or placebo. As with the measurements made over runs, however, there were many statistically significant differences between the ground reaction force patterns of the birds in the different feeding groups.

Figure 6.12: Stance Time over a step

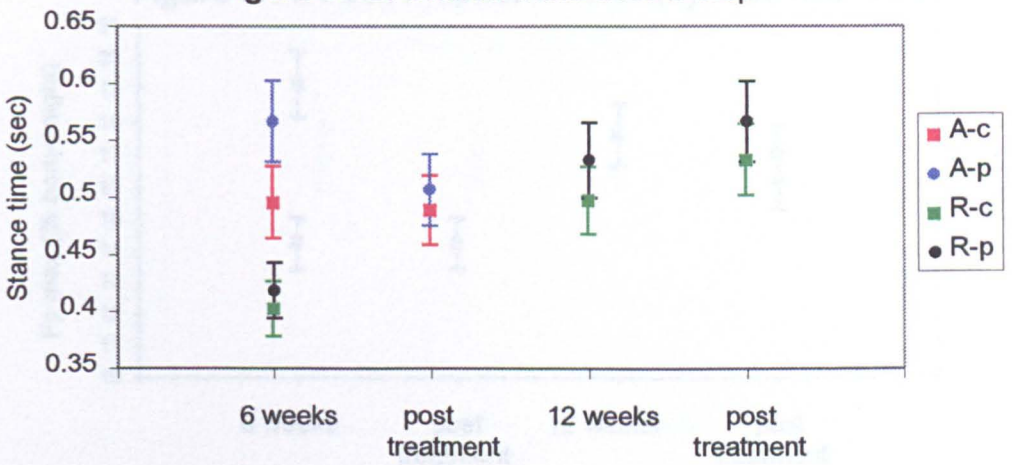


Figure 6.12: median stance time over a step (±SE median). Median stance time was significantly greater in the *ad libitum*-fed birds than the restricted-fed birds at the same age ($p<0.05$-0.01), but there was no significant difference at the same bodyweight. Median stance time was significantly greater in the restricted-fed birds at 12 weeks than at 6 weeks ($p<0.01$-0.001).

Figure 6.13: Peak Vertical Force (Fz max) over a step

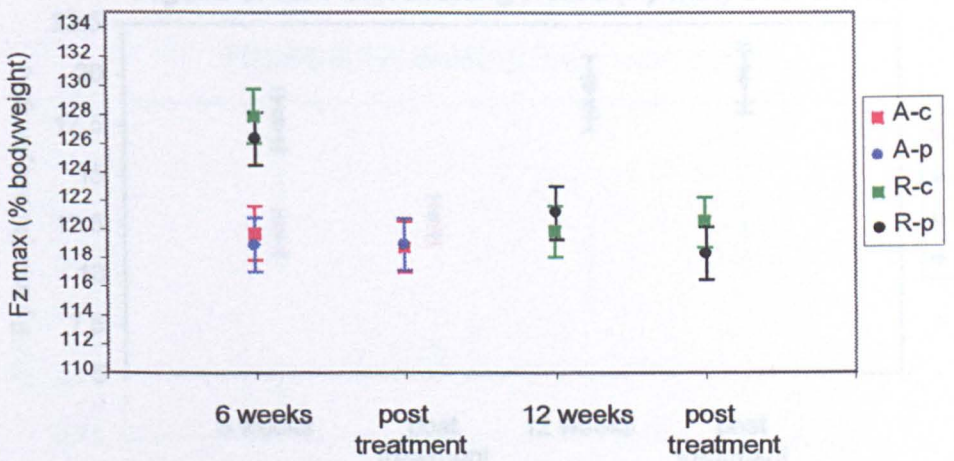


Figure 6.13: median peak vertical force over a step (± SE median). Median Fz max was significantly greater in the restricted-fed birds than the *ad libitum*-fed birds at the same age (6 weeks, $p<0.05$-0.01), but there was no significant difference at the same bodyweight. Median Fz max was significantly greater in the restricted-fed birds at 6 weeks than at 12 weeks ($p<0.05$-0.001).

Figure 6.14: Peak Propulsive Force (Fy max) over a step

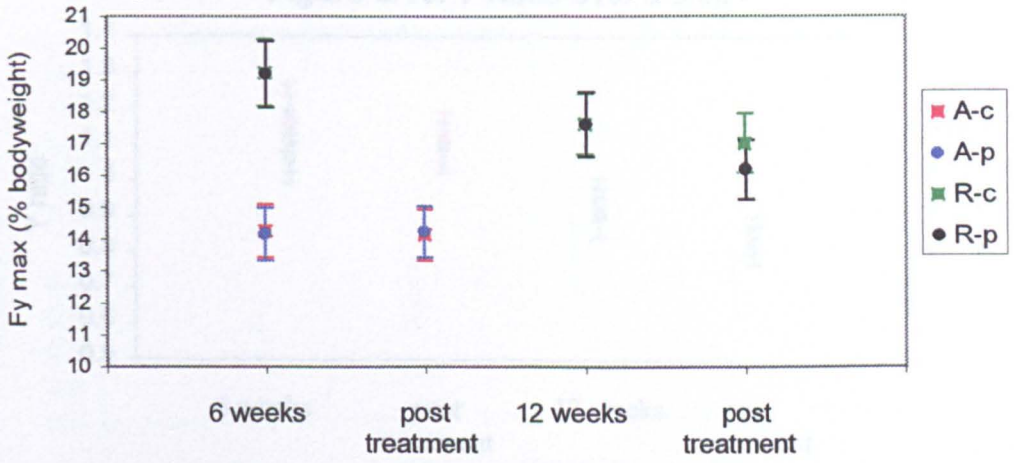


Figure 6.14: median peak propulsive force over a step (± SE median). Median Fy max was significantly greater in the restricted-fed birds than the *ad libitum*-fed birds at the same age (6 weeks, $p<0.01$-0.001), and also at the same bodyweight ($p<0.05$). There was no significant difference in median Fy max of the restricted-fed birds at 6 and 12 weeks.

Figure 6.15: Peak Braking Force (Fy min) over a step

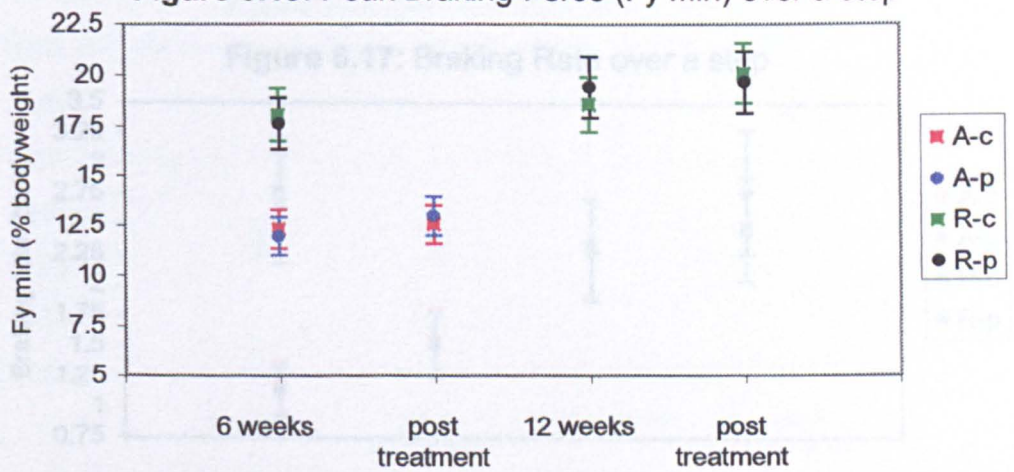


Figure 6.15: median peak braking force over a step (±SE median). Median peak braking force was significantly greater in the restricted-fed birds than the *ad libitum*-fed birds at the same age ($p<0.01$), and at the same bodyweight ($p<0.001$). There was no significant difference in peak braking force between the restricted-fed birds at 6 and 12 weeks.

Figure 6.16: Y Ratio over a step

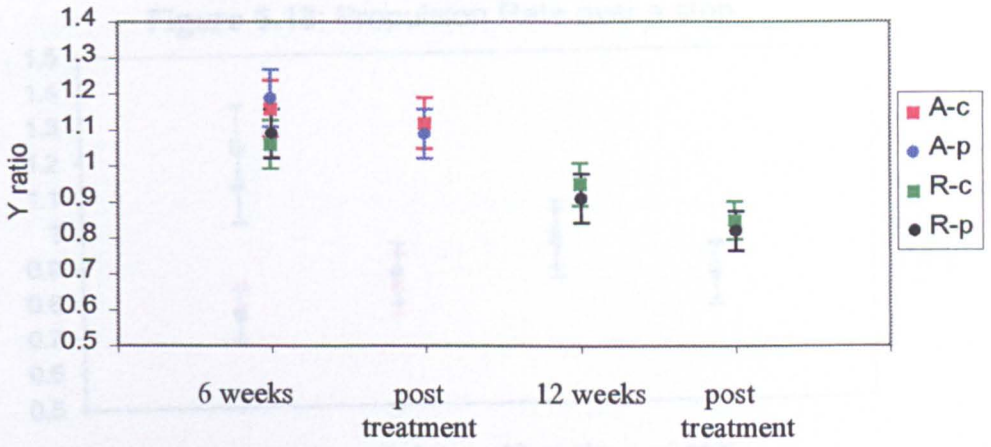


Figure 6.16: median Y ratio over a step (±SE median). Median Y ratio was not significantly different between the *ad libitum* and restricted-fed groups at the same age, but was significantly higher in the *ad libitum*-fed birds than the restricted-fed birds at the same bodyweight ($p<0.05$-0.01). Median Y ratio was significantly higher in the pre-placebo restricted-fed birds at 6 weeks compared to 12 weeks ($p<0.05$), but there was no difference between the pre- carprofen restricted-fed birds at these ages.

Figure 6.17: Braking Rate over a step

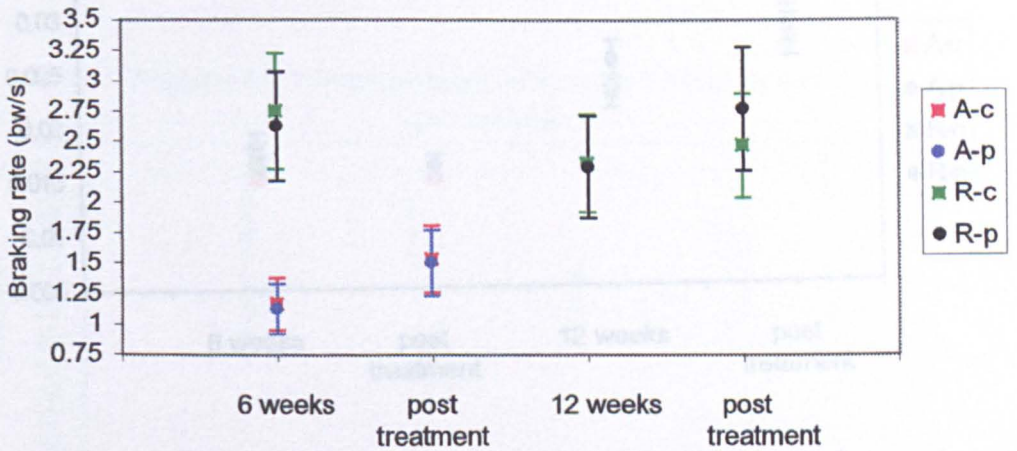


Figure 6.17: median braking rate over a step (± SE median). Median peak braking rate was significantly greater in the restricted-fed birds than the *ad libitum*-fed birds at the same age ($p<0.01$), and at the same bodyweight ($p<0.05$). There was no significant difference in median peak braking force in the restricted-fed birds at 6 weeks compared to 12 weeks.

Figure 6.18: Propulsion Rate over a step

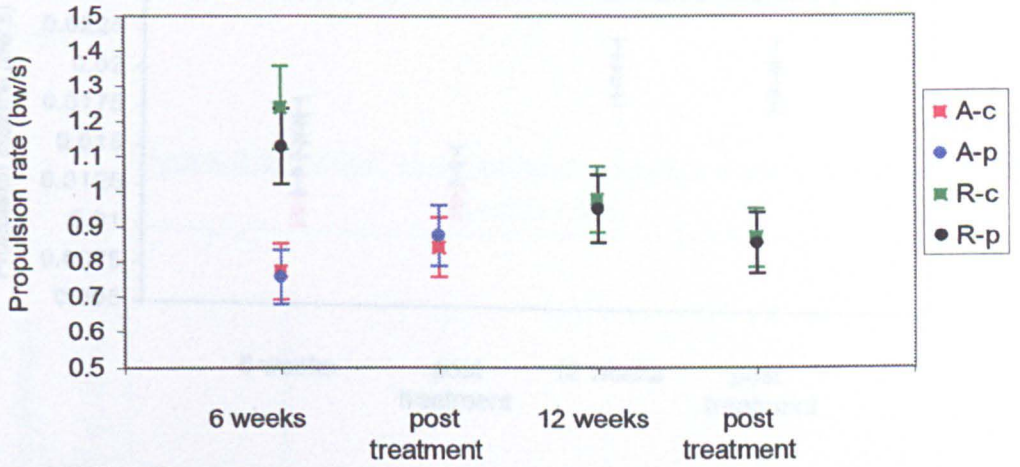


Figure 6.18: median propulsion rate over a step (± SE median). Median propulsion rate was significantly greater in the restricted than the *ad libitum*-fed birds at the same age (6 weeks, $p<0.01$), but there were no significant difference between the groups at the same bodyweight, or within the restricted-fed group at 6 compared to 12 weeks.

Figure 6.19: Braking Integral over a step

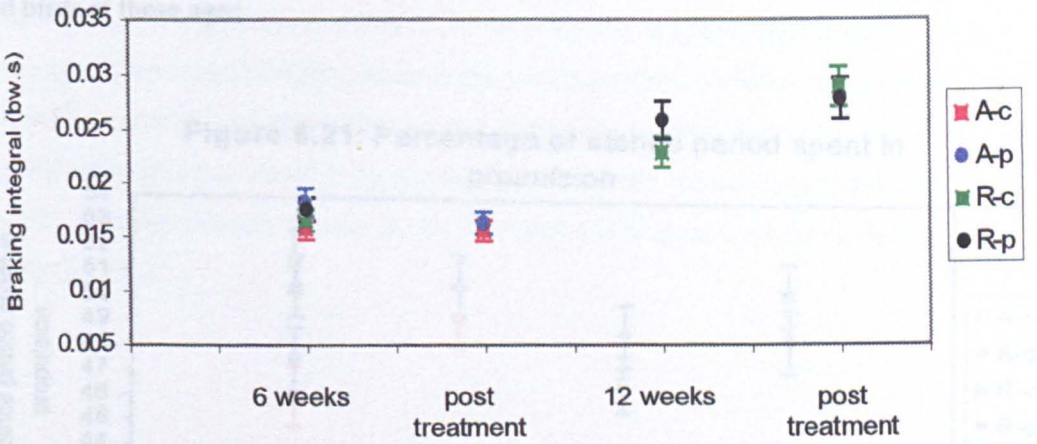


Figure 6.19: median braking integral (±SE median). Median braking integral was not significantly different between the restricted and *ad libitum*-fed birds at the same age, but was significantly higher in the restricted-fed birds than the *ad libitum*-fed birds at the same bodyweight ($p<0.001$). Median braking integral was significantly higher in the restricted-fed birds at 12 weeks than at 6 weeks ($p<0.001$).

Figure 6.20: Propulsion Integral over a step

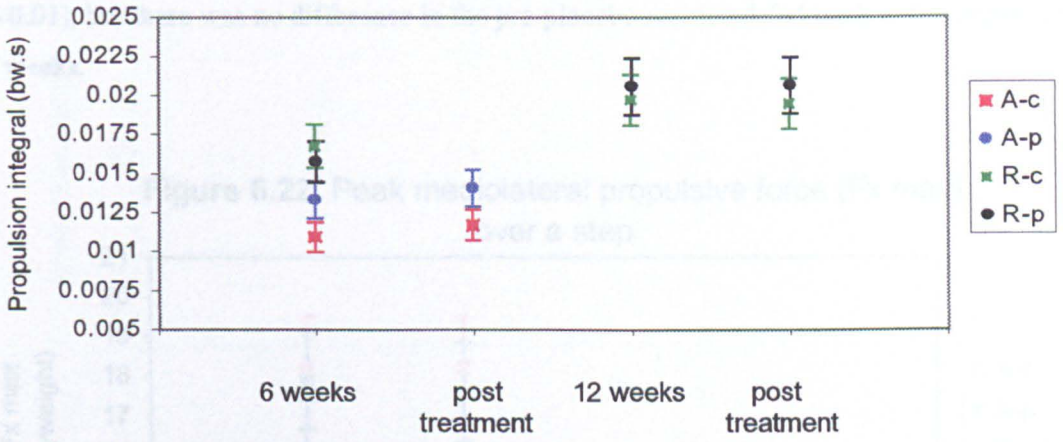


Figure 6.20: median propulsion integral (±SE median). Median propulsion integral was significantly greater in the pre-carprofen restricted-fed birds than the pre-carprofen *ad libitum*-fed birds at 6 weeks ($p<0.01$), but not between the pre-placebo restricted and *ad libitum*-fed birds. Median propulsion integral was significantly higher in the restricted-fed birds than the *ad libitum*-fed birds at the same bodyweight ($p<0.01$-0.001). Median propulsion integral was significantly greater in the pre-placebo restricted-fed birds at 12 weeks than at 6 weeks, ($p<0.05$) but there was no difference in the pre-carprofen restricted-fed birds at these ages.

Figure 6.21: Percentage of stance period spent in propulsion

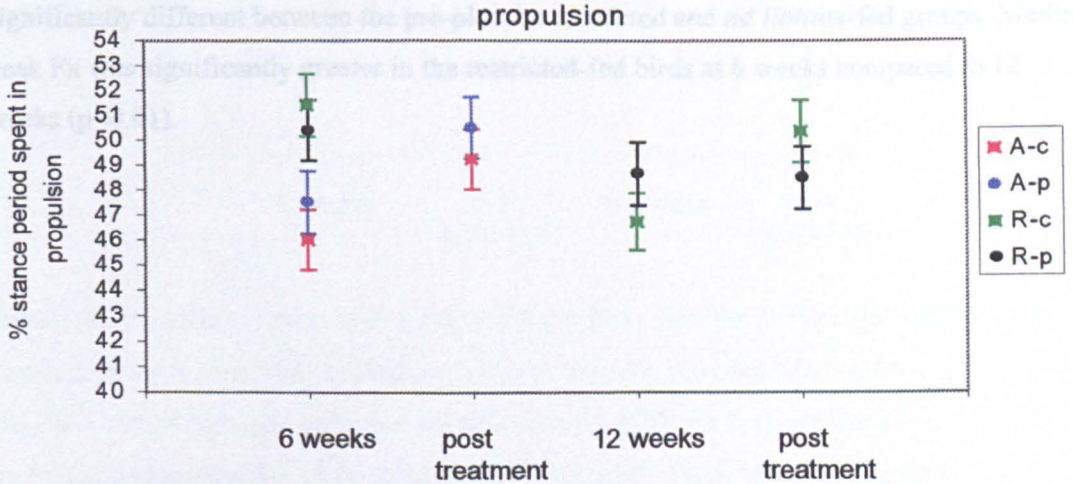


Figure 6.21: median % stance time spent in propulsion (± SE median). Median % ST spent in propulsion was significantly greater in the pre-carprofen restricted-fed birds than the pre-carprofen *ad libitum*-fed birds at the same age ($p<0.01$), but no different between the pre-placebo *ad libitum* and restricted-fed birds. There was no difference between the *ad libitum*

and restricted-fed groups at the same bodyweight. The pre-carprofen restricted-fed birds had significantly higher median % ST spent in propulsion at 6 weeks compared to 12 weeks ($p<0.01$), but there was no difference in the pre-placebo restricted-fed birds at 6 compared to 12 weeks.

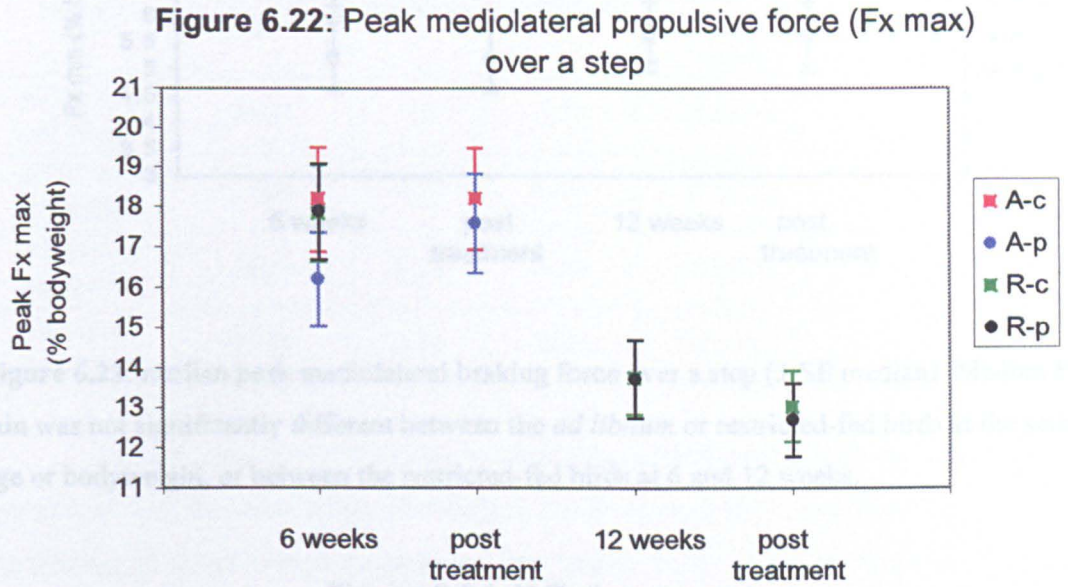


Figure 6.22: median peak mediolateral propulsive force over a step (± SE median). Median peak Fx was not significantly different between the *ad libitum* or restricted-fed birds at the same age. It was significantly greater in the pre-carprofen *ad libitum*-fed birds than the pre-carprofen restricted-fed birds at the same bodyweight however ($p<0.01$), though not significantly different between the pre-placebo restricted and *ad libitum*-fed groups. Median peak Fx was significantly greater in the restricted-fed birds at 6 weeks compared to 12 weeks ($p<0.01$).

Figure 6.23: Peak mediolateral braking force (Fx min) over a step

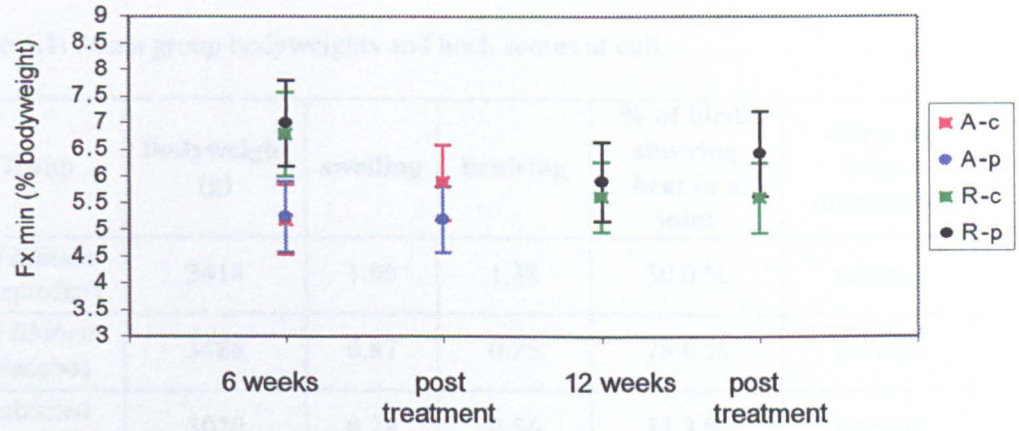


Figure 6.23: median peak mediolateral braking force over a step (± SE median). Median Fx min was not significantly different between the *ad libitum* or restricted-fed birds at the same age or bodyweight, or between the restricted-fed birds at 6 and 12 weeks.

Figure 6.24: X Ratio over a step

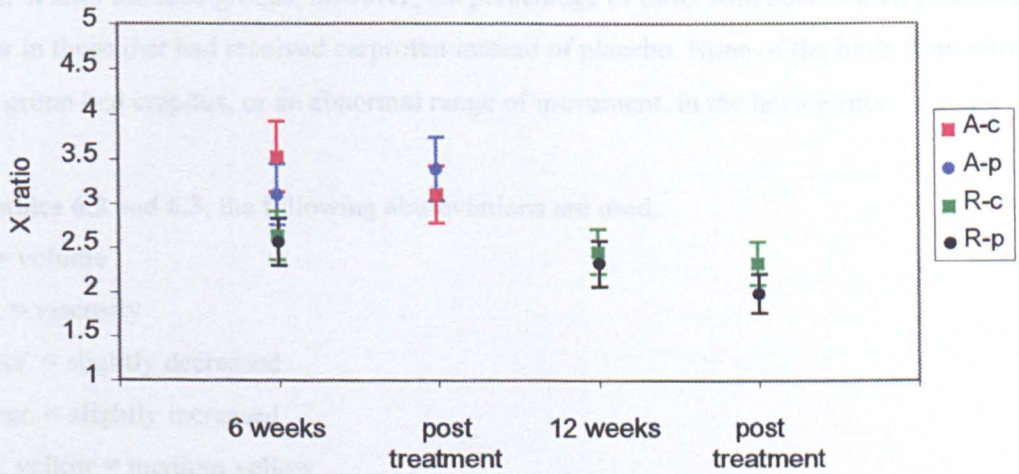


Figure 6.24: median X ratio over a step (±SE median). Median X ratio was significantly higher in the pre-carprofen *ad libitum*-fed birds than the pre-carprofen restricted-fed birds at the same bodyweight ($p<0.05$), but not significantly different between the pre-placebo *ad libitum* and restricted-fed birds at the same bodyweight. There were no significant differences between the groups at the same age or between the restricted-fed birds at 6 and 12 weeks.

HOCK SCORES AND MEASUREMENTS MADE POST-MORTEM.

Table 6.1: Mean group bodyweights and hock scores at cull.

Group	Bodyweight (g)	swelling	bruising	% of birds showing heat in a joint	range of joint movement	crepitus
ad libitum (carprofen)	3414	1.00	1.38	50.0 %	normal	-ve
ad libitum (placebo)	3486	0.87	0.75	75.0 %	normal	-ve
restricted (carprofen)	3020	0.28	0.56	33.3 %	normal	-ve
restricted (placebo)	3073	0.13	0.50	43.8%	normal	-ve

Table 6.1 shows that the mean weight of the *ad libitum*-fed birds at cull was greater, as were the mean scores for swelling and bruising of the hocks. A greater percentage of the *ad libitum*-fed birds had heat in their hocks, compared to the restricted-fed birds. Within the feed groups, however, the percentage of birds with heat in their joints was lower in those that had received carprofen instead of placebo. None of the birds from either feed group had crepitus, or an abnormal range of movement, in the hock joints.

In **Tables 6.2** and **6.3**, the following abbreviations are used:

vol = volume

visc. = viscosity

sl.decr. = slightly decreased

sl. incr. = slightly increased

med. yellow = medium yellow

n = normal

* The birds which received carprofen have a 'c' following their number, the others received placebo.

Table 6.2: Assessment of synovial fluid samples from the restricted-fed birds

Bird	Leg	Vol (ml)	turbidity	colour	Visc.	blood	fresh clots	pH	SG
18c	R	0.4	slight	pale yellow	n	-	-	8.0	1.010
	L	0.6	cloudy	pale yellow	n	-	-	7.5	1.009
1c	R	0.4	slight	pale yellow	n	-	-	7.5	1.007
	L	0.6	slight	med.yellow	n	-	-	7.5	1.008
13c	R	0.6	cloudy	med.yellow	n	old	+	8.5	1.008
	L	0.7	slight	pale yellow	n	-	-	8.0	1.008
10c	R	0.6	slight	pale yellow	n	-	-	7.5	1.008
	L	0.6	slight	pale yellow	n	-	-	8.0	1.008
14c	R	0.3	slight	pale yellow	n	fresh	+	7.5	1.008
	L	0.4	slight	pale yellow	n	-	-	7.5	1.010
15c	R	0.2	slight	pale yellow	n	-	-	7.5	1.009
	L	0.2	slight	pale yellow	n	-	-	7.5	1.009
16c	R	0.5	clear	clear	n	-	-	7.5	1.007
	L	0.6	slight	pale yellow	n	-	-	7.5	1.007
4c	R	0.5	cloudy	med.yellow	n	old	+	7.5	1.007
	L	0.6	cloudy	med.yellow	n	-	-	7.5	1.008
7c	R	0.6	cloudy	pale yellow	n	-	+	7.0	1.010
	L	0.6	clear	clear	n	-	-	7.0	1.007
8	R	0.8	slight	pale yellow	n	-	-	8.0	1.008
	L	0.9	clear	pale yellow	sl.decr.	-	-	8.0	1.007
6	R	0.7	slight	pale yellow	n	fresh	+	7.5	1.007
	L	0.5	slight	pale yellow	n	fresh	+	7.5	1.009
20	R	0.5	slight	pale yellow	n	-	-	7.0	1.009
	L	0.5	clear	clear	n	-	-	8.0	1.008
11	R	0.7	slight	pale yellow	n	-	-	7.5	1.007
	L	0.8	cloudy	Pink/ yellow	n	old	-	7.5	1.009
17	R	0.6	slight	pale yellow	n	fresh	+	7.0	1.007
	L	0.8	clear	clear	n	-	-	7.0	1.007
5	R	0.8	cloudy	med.yellow	sl.decr.	-	-	7.5	1.008
	L	0.8	bloody	bloody	n	old	-	8.0	1.009
12	R	0.3	slight	pale yellow	n	-	-	8.0	1.010
	L	0.3	cloudy	med.yellow	n	fresh	+	8.0	1.010
19	R	0.5	slight	pale yellow	n	-	-	8.0	1.009
	L	0.4	slight	pale yellow	n	fresh	+	7.5	1.008
Summary of results, showing median values, and percentages of all samples :									
Birds given carprofen		0.60	88.9 % turbid	11.11 % with 'old' blood	mostly normal		22.2%	7.5	1.008
Birds given placebo		0.65	81.2 % turbid	12.5 % with 'old' blood	mostly normal		31.2%	7.5	1.008

Table 6.2 shows that most of the joint fluid samples from the hocks of the restricted-fed birds were slightly cloudy, and that approximately 12% of the samples contained old blood, and 22 - 31% had fresh blood clots present. There was no difference in median pH or SG between the synovial fluid of those birds that received carprofen or placebo.

Table 6.3: Assessment of synovial fluid samples from *ad libitum*-fed birds

Bird	Leg	Vol (ml)	turbidity	colour	Visc.	blood	fresh clots	pH	SG
21c	R	0.3	cloudy	very bloody	n	+	+	9.0	1.009
	L	0.3	cloudy	very bloody	n	+	+	8.5	1.010
30c	R	0.3	cloudy	bloody	n	+	+	8.5	1.013
	L	0.2	cloudy	bloody	n	+	+	8.5	1.012
32c	R	0.4	cloudy	very bloody	n	+	+	8.5	1.010
	L	0.5	cloudy	bloody	sl.decr	+	+	9.0	1.009
38c	R	0.2	cloudy	med.yellow	n	-	trace	8.0	1.011
	L	0.3	cloudy	med.yellow	n	-	-	8.0	1.012
31c	R	0.6	slight	pale yellow	n	-	trace	8.0	1.010
	L	0.5	slight	pale yellow	n	-	trace	8.0	1.010
22c	R	0.5	cloudy	pinkish	sl.decr	+	+	8.5	1.009
	L	0.8	cloudy	very bloody	n	+	+	8.5	1.010
23c	R	0.3	cloudy	pale yellow	n	-	-	8.0	1.012
	L	0.4	cloudy	pinkish	n	+	trace	8.0	1.009
26c	R	0.4	cloudy	pale yellow	n	-	-	8.3	1.010
	L	0.4	cloudy	med.yellow	n	-	+	8.3	1.010
28	R	0.4	cloudy	bloody	n	+	+	8.0	1.011
	L	0.4	cloudy	med.yellow	n	-	+	8.0	1.012
33	R	0.3	slight	pale yellow	n	-	-	8.0	1.010
	L	0.5	cloudy	medium yellow	n	-	-	8.0	1.012
36	R	0.5	cloudy	slightly bloody	n	+	+	8.0	1.010
	L	0.4	cloudy	bloody	n	+	+	8.3	1.010
27	R	0.3	cloudy	very bloody	n	+	+	8.3	1.012
	L	0.4	cloudy	bloody	n	+	-	8.3	1.012
40	R	0.4	cloudy	very bloody	n	+	+	8.5	1.010
	L	0.3	cloudy	very bloody	sl.decr	+	+	8.5	1.009
39	R	0.2	cloudy	medium yellow	sl.incr	-	-	8.0	1.011
	L	0.4	cloudy	medium yellow	n	+	-	8.0	1.011
37	R	0.3	blood	bloody	n	+	+	8.3	1.008
	L	0.4	cloudy	very bloody	n	+	-	8.3	1.009
35	R	0.3	cloudy	recent haem.	n	+	+	8.5	1.009
	L	0.6	slight	pale yellow	n	-	-	8.0	1.012
Summary of results, showing median values, and percentages of all samples :									
Birds given carprofen		0.4	100 % turbid	56.25 % with 'old' blood	mostly normal		81.2%	8.40	1.010
Birds given placebo		0.4	100 % turbid	62.5 % with 'old' blood	mostly normal		56.2%	8.15	1.011

Table 6.3 shows that all the samples of joint fluid from the hocks of the *ad libitum*-fed birds were cloudy, and that a large number contained both old blood, and fresh blood clots. This is reflected in the increased specific gravity of the samples. The pH of the synovial fluid was also quite alkaline. There does not appear to be much difference between those birds that received carprofen rather than placebo, except that the percentage of joints containing fresh blood was considerably lower in the post-placebo group.

BONE ANALYSIS

Tibiotarsal torsion: none of the restricted-fed broilers had abnormal lateral rotation of the tibiotarsus, but ten of the birds had a medial torsion in one limb. Only one of these was greater than 5 degrees, however (5.16 degrees). In the *ad libitum*-fed group, 6 of the birds had unilateral medial torsion of the tibiotarsus, and one bird had bilateral medial rotation, however only one case was greater than 5 degrees (6.16). There appeared to be no difference in incidence based on whether the birds received carprofen or placebo.

Tibial plateau angle (TPA): six of the restricted-fed birds had TPA's of 26-27 degrees, compared to only one bird from the *ad libitum*-fed group. Again, there appeared to be no difference based on drug treatment.

Histological examination of the proximal epiphyseal sections: one bird from the *ad libitum*-fed (carprofen) group had evidence of tibial dyschondroplasia in the proximal epiphysis of the right tibiotarsus (bird 26c, right leg). The measurements for tibiotarsal torsion and tibial plateau angle in the bone were normal however.

6. 4 DISCUSSION

In the following discussion, comparisons are made between the GRF's of the birds raised under the different feeding regimes, to test the hypothesis that growth rate influences the ground reaction force patterns in broilers. The results are also compared to those of the Brown Leghorns in the previous experiment (Chapter 5). The second hypothesis, that the gait of the rapidly growing *ad libitum*-fed birds may be affected by the presence of pain, is tested by comparing the gait patterns before and after administration of an analgesic drug. For the purpose of the first part of the discussion, it is assumed that carprofen, at the given dose and route of administration, is an effective analgesic in broilers. This may not be the case of course, and other possibilities are discussed at the end.

In the previous experiment on Brown Leghorns, speed was shown to have a significant effect on many aspects of the ground reaction forces. This indicated that the GRF data should be analysed in the context of particular speed ranges, and in the previous experiment, three speed ranges were derived. In the present experiment, the median speeds were all within the 'slow' range as defined by the Brown Leghorn data (0.153-0.409m/s). Thus all the data from the broilers can be considered within a single speed group, and comparisons made with Brown Leghorn data from the slow speed range.

In contrast to previous work in this thesis, male birds were used in the present experiment. The more intensive genetic selection and greater production stresses placed on males results in a higher incidence of musculoskeletal disease than in females (Hocking, 1992). Although clinically lame birds were not used, it was thought that males would be more lightly to suffer from sub-clinical lameness, and would therefore show a more marked response to analgesia. The fact that the GRF's are normalised to bodyweight should minimise any effects of sexual dimorphism, allowing comparisons to be made with results from female birds.

SPEED and CADENCE:

As demonstrated in previous work in this thesis, restricted-fed broilers tend to show significantly higher speeds than *ad libitum*-fed birds, both at the same age, and the same bodyweight. Although this was confirmed in the present study, it is notable that all the broilers moved at speeds similar to 'slow' Brown Leghorns. The significantly greater median cadences of the restricted-fed broilers compared to the *ad libitum*-fed birds at the same age can be accounted for by the higher speeds shown by the former birds. Comparing the results of the restricted-fed birds at 6 and 12 weeks, their speed was variable, but their cadence was significantly lower by 12 weeks, which could be explained by the birds taking longer steps. A decrease in cadence with age has also been demonstrated in children (Sutherland *et al*, 1988), the higher cadence in the young being suggested as a method of compensating for their shorter stride lengths (Todd *et al*, 1989).

A lame subject will usually walk more slowly than a sound one, speed often varying with the degree of lameness (Andriacchi *et al*, 1977; Steven *et al*, 1983). Had the birds in the present study been in pain, their walking speed might have increased post-analgesic, which was not the case. Although there was a statistically significant increase in the speed of the *ad libitum*-fed birds given the placebo, there is no obvious biological reason for this (and a similar placebo effect was not apparent in the restricted-fed group).

STANCE TIME:

Stance times measured over runs represent the time taken for the bird to cross the plate (usually taking several steps), and so vary with speed. It is not surprising, therefore, that median stance time for the runs and the individual steps was significantly greater in the *ad libitum*-fed birds than the restricted-fed birds at 6 weeks, reflecting the slower speeds of the former group. Although the median stance times for the runs were significantly greater in the *ad libitum*-fed birds than the restricted-fed birds at the same bodyweight, there was no

significant difference in stance time of the individual steps. This suggests that the *ad libitum*-fed birds took more steps to cross the plate (and so these steps would have been shorter). Comparing the stance times of the restricted-fed birds at 6 and 12 weeks, there was no significant difference the stance time of the runs, however there was a significant increase in individual step stance time. As speed did not change significantly, this would suggest that the step length became longer, as would be expected with their increasing leg length. The tendency of the *ad libitum*-fed birds to take short steps, and the restricted-fed birds to take longer ones, is in agreement previous findings in this thesis (Chapter 4).

The effects of lameness on stance time can be more variable. If the problem is bilateral, the subject will tend to move more slowly and therefore the stance periods will increase. If the subject has a single painful limb, however, it is likely to show a typical antalgic gait: short stance periods on the painful limb, and longer ones on the sound limb (Whittle, 1991). In the present experiment, administration of analgesic did not result in a significant change in stance time over steps or runs.

VERTICAL LOADING RATE:

The vertical loading rates in the present experiment were very variable, which was not unexpected, previous work on Brown Leghorns having shown that this parameter is significantly affected by speed. Both the restricted and *ad libitum*-fed broilers showed considerably lower rates of change of vertical force than the Brown Leghorns (6.5 bw/s), even although they were compared within the same speed range. The vertical loading rate is measured for the first step only, and so it is possible that the broilers may have been more hesitant than the Brown Leghorns in taking that first step onto the plate. Another explanation may be that the birds were flexing their legs slightly during loading, which would have effectively decreased the loading rate. Flexing the joints of the limb is the most effective way of decreasing impulsive forces during walking (Collins and Whittle, 1989), and it may be that the broilers make more effective use of this than the Brown Leghorns. It has also been shown that vertical loading rate decreases in lameness (Budsberg *et al*, 1988; Ghosh *et al*, 1993; Rumph *et al*, 1993), however analgesia had no significant effect on loading rate in the present experiment.

PEAK VERTICAL FORCE:

a) measured over a run:

The peak vertical force over a run must equal or exceed the bodyweight of the bird as it walks across the plate. As the forces are presented as percentages of bodyweight, it is interesting that the median peak vertical force was significantly greater in the restricted-fed birds (133.93-134.17 % bw) than the *ad libitum*-fed birds (124.59-126.75 % bw) at 6 weeks. This could be explained by the higher speed of the restricted-fed birds, as discussed in Chapter 5. On reaching a similar bodyweight, however, there is no significant difference in peak vertical force between the *ad libitum* and restricted-fed birds, and the forces are similar to those seen in Brown Leghorns (125% bw). This suggests that the birds may use similar mechanisms to control the excursions of the CG in a vertical direction, resulting in a dynamically similar gait (within the range 120-150% bw demonstrated to occur in human walking – Betts *et al*, 1980a; Whittle, 1991).

The fact that the restricted-fed birds' peak vertical forces decreased with age, despite speed being similar, is interesting. The vertical force is basically a product of bodyweight, and the vertical acceleration and deceleration as the CG falls downwards (as well as forwards) in the latter half of the stance phase. As expressing the forces as percentages of bodyweight effectively controls for differences in bodyweight, the increase the vertical force must be due to an increase in the vertical acceleration and deceleration of the CG. It therefore seems likely that the restricted-fed birds changed their gait as they grew, reducing the vertical excursions of the CG e.g. perhaps chicks take more 'bouncy' steps than the adult birds. The *ad libitum*-fed chicks, putting on massive amounts of weight very rapidly, may not have the same 'spring' in their step.

The lower speeds and longer stance periods of the *ad libitum*-fed birds in this study agree with the findings of Chapter 4, where it was suggested that the increased stance times, allowing the load (bodyweight) to be spread over a longer period of ground contact, would reduce the peak vertical forces on the skeleton. The results of the present experiment confirm this. Thus the stresses on the immature bones are lower, in keeping with the aim of maintaining the stresses within 'safe' limits (Biewener *et al*, 1986; Frost, 1997).

If a limb is painful, the peak vertical forces are usually reduced, as the subject avoids loading the full bodyweight onto the leg. This has been demonstrated in humans, who in extreme cases use canes or crutches to transmit the forces through the arms rather than a limb (Whittle, 1991). Various other studies have demonstrated a reduction in peak vertical force with lameness in horses (Merkens and Schamhardt, 1988a), sheep (Ghosh *et al*, 1993) and dogs (Budsberg *et al*, 1988; Rumph *et al*, 1993). In some cases, a significant

inverse correlation has been shown between the clinical grade of lameness and the peak vertical force (and impulse) (Jevens *et al*, 1996). Other studies on quadrupeds have demonstrated that a reduction in peak vertical forces in one limb results in compensatory increases in the contralateral forelimb and ipsilateral hindlimb (Merkens and Schamhardt, 1988b; Rumph *et al*, 1993; Jevens *et al*, 1996). As birds take several steps across the plate, measurement of peak vertical force in a lame bird over a run may therefore produce higher values than those of a sound bird. For this reason, it would be important to look for changes in this particular parameter over individual steps.

b) measured over a step:

Some of the peak vertical forces produced during a step were less than 100% of bodyweight, indicating that on those particular steps, the whole of the bird's weight was not loaded onto the stance leg. As the birds were not seen to stumble, the most likely explanation is that the joints of the limb flexed slightly as the leg began to weightbear, temporarily unloading it, similar to the way the minima is produced by slight knee flexion during mid-stance in human walking (Whittle, 1991). Most of these were single occurrences for a bird, however, and the forces remained above 90 % bw. These single episodes are unlikely to be of biological significance, as had the birds been obviously lame, the values should be consistently much lower, over several runs from the particular bird (unless, of course, the step of the sound limb was measured). However, this does confirm that the peak % bodyweight should be analysed for data from individual steps, rather than peak % bodyweight -100. With data from runs, however, peak vertical force must be equal to or greater than bodyweight (100%), and peak%-100 can therefore be used, as it gives a more normal distribution. (As discussed in Chapter 2.5, it is much more important to have a normal distribution when establishing 95% ranges for parameters in 'normal' birds, than to make simple comparisons between averages).

As none of the birds appeared to be lame, it was not unexpected to find that the peak vertical forces were not significantly different over a step as compared to a run, and did not change significantly following analgesia.

BRAKING AND PROPULSIVE FORCES:

a) measured over a run:

The peak braking and propulsive forces were significantly greater in the restricted-fed birds than the *ad libitum*-fed birds at 6 weeks, which is not surprising as the former were moving at higher speeds. Within the groups at 6 weeks, the braking and propulsive forces

were approximately equal, as would be expected in a subject moving at a steady speed. In the Brown Leghorn data, the ratio of propulsion to braking was 26: 20 (Y ratio of 1.28), which suggests that the Leghorns were actually accelerating over the plate. In the present experiment, the peak propulsive forces decreased in the older restricted-fed birds, however the braking forces remained similar, and so the Y ratio was less than one. This suggests that the older birds showed an overall deceleration across the plate. It is possible that these older birds had learned to associate the person at the end of the runway with receiving their food ration, and so were less inclined to move away. This again illustrates how strongly motivation can influence gait in birds. The effects of motivation on gait have also been demonstrated in other species, where parameters such as limb positioning and timing were shown to change depending upon whether the animal was exposed to an aversive stimulus, or a food reward (Wetzel and Stuart, 1977).

b) measured over a step:

The median braking and propulsive forces were significantly greater in the restricted-fed birds than the *ad libitum*-fed birds (at both the same age and the same bodyweight), as would be expected from the faster speeds (and significantly shorter stance times) of the former birds. The Y ratios were slightly greater than one for the younger birds of both groups (at 6 weeks), which indicates that they moved at a reasonably steady speed across the plate. In the older restricted-fed birds, the propulsive force decreased significantly, but the braking force did not change, and so the Y ratio became less than one.

There was no significant change in the craniocaudal forces following analgesia. The results of Chapter 5 suggest that the peak craniocaudal forces will be lower if the lameness causes the bird to move more slowly. Other studies have found the effect of lameness on craniocaudal forces to be very variable, however. A study on forelimb lameness in horses showed the greatest reduction in left-right symmetry was in the craniocaudal peak forces (Merkens and Schamhardt, 1988a). These authors also demonstrated that decreased braking forces in a lame hindlimb are mainly compensated for by increased propulsive forces in the contralateral hindlimb (Merkens and Schamhardt, 1988b). Whether the Y ratio would change is also difficult to predict; it seems likely that the braking forces would be greater than the propulsive forces if the animals are inclined to slow down (through pain or fatigue). However, the present experiment has demonstrated that similar effects can occur depending on the motivation of the birds.

BRAKING AND PROPULSIVE RATES AND INTEGRALS:

At 6 weeks, the higher peak craniocaudal forces and faster speeds of the restricted-fed birds resulted in them having significantly greater median propulsion and braking rates than the *ad libitum*-fed birds. In comparison, the Brown Leghorns had similar braking rates (1bw/s) to the *ad libitum*-fed broilers (1.2-1.5 bw/s). The propulsion rates of the Brown Leghorns were much greater than either group of broilers, however, suggesting that they were moving faster across the plate. Thus although the birds were considered within the same 'slow' speed range, it is possible that more of the Brown Leghorns moved at speeds near the top of this range, compared to the broilers. There was no significant difference in propulsion rate between the *ad libitum* and restricted-fed birds at the same bodyweight, however the stance time was significantly greater in the *ad libitum*-fed birds, resulting in a significantly greater propulsion integral in these birds.

Median peak braking rate was significantly greater in the restricted-fed birds than the *ad libitum*-fed birds at the same bodyweight, and so was the median peak braking integral (and peak braking force). In the Brown Leghorns, the median braking integral (0.0152 bw.s) was similar to that of both food groups around 6 weeks, but lower than that of the older restricted-fed birds (0.0229-0.0289 bw.s). In contrast, the propulsion integrals were similar between the broilers (at all stages) and the Brown Leghorns. Comparing the restricted-fed birds at 6 and 12 weeks, the only consistent finding was a significantly higher median braking force and integral at 12 weeks, which might be expected if the birds were indeed decelerating across the plate.

The effect of lameness on braking and propulsive impulses has been found to be variable. In normal walking, if the full bodyweight is taken onto a limb, a shorter stance period will result in a higher peak force. Where the limb is painful, however, various compensatory mechanisms are used to reduce the loading, and the stance period is also reduced (Whittle, 1991), therefore the integral may be smaller. This was demonstrated in a study of lame dogs by Jevens *et al* (1996), which showed decreased braking and propulsive impulses with lameness. Other authors reported no differences in peak braking force or impulse with lameness, but a significant negative correlation between hindlimb peak propulsion force and impulse with lameness (Budsberg *et al*, 1996). In the present study, the median braking integral was found to increase significantly in the restricted-fed birds following carprofen. In isolation, however, this is unlikely to be significant in a biological sense.

% STANCE TIME :

In the present study, the % stance time spent in propulsion ranged from 46.04-51.44%, which is similar to the value for the Brown Leghorns (48.8%). This shows that broilers behave similarly to other species in the fact that the proportion of the step spent braking vs propelling is unaffected by speed (Wetzel and Stuart, 1977; McLaughlin and Roush, 1995). It seems likely, therefore, that this particular measurement would also be unaffected by lameness.

MEDIOLATERAL FORCES:

It is important to consider how the mediolateral forces differ over an individual step, compared to a run. Over a run, several consecutive steps are measured, and so the 'maximum' and 'minimum' forces are actually maximum forces in opposite directions, alternating between consecutive steps. During a single step, however, most of the time the foot is on the ground it is accelerating the CG towards the opposite foot (Whittle, 1991). Thus the maximum force is the larger propulsive force which 'pushes' the body mass over onto the other leg i.e. in the direction in which the body is moving (hereafter called the 'mediolateral propulsive force'). The minimum force is the smaller braking force, as the other foot contacts the ground, and so can be considered as the 'mediolateral braking force'. Thus as a foot is placed on the ground, it first produces a 'small' braking force which falls to zero as the CG is positioned over the stance leg; it then produces a 'larger' propulsive force to push the CG back as the other leg takes over the stance function. Although mediolateral forces are usually ignored in gait analysis of most species (for being small and variable), they were shown to be of large size in the Brown Leghorns (10.4-10.9 % bw). These forces were even larger in the broilers, as might have been predicted from the exaggerated lateral sway seen as these birds walk.

a) measured over a run:

There was no significant difference in median mediolateral force between the restricted and *ad libitum*-fed birds at 6 weeks (16.95 – 20.78% bw), but they were up to twice as large as those of the Brown Leghorns (10.4-10.9%bw). At the same bodyweight, however, they were significantly higher in the *ad libitum*-fed birds than the restricted-fed birds, as by 12 weeks, the forces in the restricted-fed birds had decreased (12.97-14.63% bw).

It is not surprising that the forces were much higher in the 6 week old broilers than the Brown Leghorns, if we consider how these birds walk. Previous work in this thesis

established mean step widths of approximately 0.03m for the Brown Leghorns, and 0.07-0.08 m in selected broilers at cull, suggesting a considerably wider walking base in the latter birds. Larger forces are therefore required to produce the greater lateral excursions of the CG (to position it over the stance leg): the mediolateral forces in the younger birds are of a similar size to the craniocaudal forces. The fact that these young birds are having to producing as much force in a mediolateral direction, to shift their weight from one leg to the other, as they are to move their body mass forwards, identifies (and quantifies) another particularly inefficient aspect of broiler gait.

It was surprising to find that the mediolateral forces in the restricted-fed birds decreased significantly as the birds grew, approaching similar levels to those of the Brown Leghorns. This is despite the fact that the conformation of the restricted-fed birds was becoming more like that of the *ad libitum*-fed birds, as they increased in weight and girth and began to take wider steps. Although the conformation of the birds grew 'wider', however, their legs were also considerably longer, which means that the body was more in proportion. If a bird stands with both feet on the ground, a line drawn from the CG down to the foot will be at a more acute angle to the leg if the leg is longer, rather than shorter. The CG therefore has to move through a smaller angle to reach a position above the stance leg in a longer legged subject, which means the vertical excursion will also be less, and therefore less force is involved in moving it. It is also possible that the restricted-fed birds develop certain gait optimisations with age, as has been demonstrated with children (Sutherland *et al*, 1988).

As only straight runs were included in the analysis, it is not surprising that the X ratios were close to one (as with the Brown Leghorns - 1.04), and that there were no significant differences between the groups or ages.

b) measured over a step:

There was no difference in mediolateral propulsive and braking forces between the birds at the same age or bodyweight, with one exception: the median mediolateral propulsive force was statistically significantly greater in the pre-carprofen *ad libitum*-fed group than the restricted-fed group. Again, in isolation, this finding is unlikely to be of any real biological significance. In the restricted-fed birds, the median mediolateral propulsive force was significantly greater at 6 than 12 weeks, but there was no significant change in the median mediolateral braking force.

While the X ratios over a run must approximate 1 (if the bird is moving in a straight line), the X ratio for a step gives an indication of how the mass of the bird is moved by the

leg in ground contact. At all stages, the X ratios were higher in the *ad libitum*-fed birds (around 3-3.5) than the restricted-fed birds (around 2-2.5). This indicates that it was the greater propulsive forces required to push the CG over onto the opposite leg which accounted for the higher values of mediolateral forces seen over a run. This would seem logical in view of the wider walking base. This ratio could be a useful measure of lameness, as the lame leg might be expected to produce lower propulsive forces. In the present study, however, analgesia had no significant effect on either mediolateral force.

POST-MORTEM ANALYSIS:

Although none of the birds appeared to be lame on visual assessment, the limitations of subjective gait analysis have already been discussed. In this experiment, synovial fluid samples were collected from the hock joints of the birds, to provide a simple but objective measure of the condition of one aspect of their musculoskeletal system. Although sampling from one joint provides limited information, gross examination of the fluid can often give a useful indication of underlying processes occurring within the joint, and can easily be used in living subjects. Analysis of the synovial fluid samples produced several unexpected results. The samples from the *ad libitum*-fed birds were consistently cloudy, and most surprisingly, 56-62.5% of the samples contained old blood, which is suggestive of previous trauma to the joints. An episode of acute, severe trauma usually causes noticeable lameness, and is unlikely to have occurred in such a high percentage of the birds. It therefore seems more likely that the bleeding occurred as a result of low-grade repetitive trauma. Although occasional bleeding can occur due to needle trauma during sampling, it was also surprising to find that over 80% of the samples from the *ad libitum*-fed birds contained fresh blood. This very high percentage could indicate that the synovial membrane was particularly vascular (probably due to inflammation) in these birds. Administration of carprofen, which has anti-inflammatory properties, appeared to have little effect on the percentage of birds with blood in their joints however. Amongst the restricted-fed birds, 11-12.5% had evidence of old blood in their joints, which is still a surprisingly high number, however the percentage with fresh blood was at a more expected level, perhaps because the synovium was less inflamed. This tends to suggest that the birds joints are being damaged during normal walking (particularly those of the *ad libitum*-fed birds), which raises considerable welfare concerns.

The second interesting finding is the alkalinity of the synovial fluid, particularly of the *ad libitum*-fed birds. Normal synovial fluid pH is between 7-7.8 in most species (Perman, 1980), and it is interesting to speculate that the marked alkalinity may be a

predisposing factor in the relatively common problem of urate arthritis in poultry. This occurs when crystals of sodium urate precipitate out in the joints, and cause a severe and painful inflammatory condition (Gentle and Corr, 1995). These interesting findings prompted further investigation of the synovial fluid, which is described in detail in the following chapter.

In common with the findings of chapter 4, and those reported by (Duff and Thorp, 1985a), medial tibiotarsal torsion was common in the *ad libitum*–fed broilers. In the present experiment, however, the incidence of both medial tibiotarsal torsion and increased tibial plateau angle was actually greater in the restricted-fed birds. In all cases, however, the values were only slightly above the normal range (torsions up to 6.6 degrees, and TPA's up to 27 degrees). Evidence of unilateral tibial dyschondroplasia was present in one *ad libitum*-fed bird, but the measurements for tibiotarsal torsion and tibial plateau angle in the bone were normal. None of the birds in the aforementioned cases were lame.

EFFECTS OF DRUG TREATMENTS:

Administration of carprofen or placebo had no effect on most of the gait parameters measured in this experiment, only three changing significantly post-treatment. There was a significant increase in speed of the *ad libitum*-fed birds following placebo administration, and the median braking integral and mean % stance time spent in propulsion were significantly greater in the restricted-fed birds following administration of carprofen. Although these differences were statistically significant, it is unlikely that they are of any real biological significance occurring in isolation. There is no obvious explanation as to why the *ad libitum*-fed birds would have shown increased speeds after placebo administration, and this placebo effect does not occur in the restricted-fed birds that received the same treatment. As the median braking integral is the product of braking force x time, if the integral increased, and braking force did not change significantly, nor did the braking rate, it must have been the time. An increase in stance time normally occurs when speed is reduced, and as has been shown repeatedly throughout this thesis, speed is strongly influenced by motivation and is therefore difficult to control. It is more difficult to explain the significant increase in the mean % stance time spent in propulsion, as previous work has shown that the time spent in propulsion or braking is usually approximately equal, and unaffected by speed. This may be an example of a Type 1 error, where the null hypothesis has been rejected in error.

There are several possible explanations as to why carprofen did not appear to have a biologically significant effect on the birds' gait:

a) carprofen may not be an effective analgesic in broilers – the pharmacological activity and efficacy has not been validated in birds.

b) the dose and, or, method of administration of the drug may have been inappropriate in broilers.

c) the gait of the birds may have been primarily determined by their conformation, irrespective of whether they were in a degree of pain (which may or may not have been affected by the carprofen), or completely pain-free.

d) the gait parameters measured in this experiment may not change in the presence of pain

e) the motivation of the birds to walk may have been the primary determinant in the observed gait patterns.

It is clear from this experiment that the ground reaction forces in broilers differ in certain ways from those of Brown Leghorns, and can be influenced by growth rate. The possible role of pain remains to be determined, however the incidental finding that more than 1 in 2 of the *ad libitum*-fed birds had blood in their joints raises serious welfare concerns.

The results from the present experiment compliment those of Chapter 4 (on morphology and gait) in supporting the hypothesis that the conformation of the birds, with the resulting biomechanical constraints, is the major factor influencing the observed gait patterns in broilers.

CHAPTER 7:

SYNOVIAL FLUID ANALYSIS OF POULTRY

7.1: INTRODUCTION

In the previous experiment, a large number of the synovial fluid samples from the broilers contained blood. This was a surprising finding, which has not been previously described in the literature. Synovial fluid analysis is not widely reported as a method of investigating joint disease in poultry, problems usually being diagnosed by *post-mortem* examination of the bones and associated tissues. This is in contrast to most other species, where synovial fluid analysis is used routinely to investigate joint physiology and pathology e.g. dogs (Sawyer, 1963; Fernandez *et al*, 1983; Houlton, 1994), horses (Lumsden *et al*, 1996; McIlwraith and Trotter, 1996) and humans (Fawthrop *et al*, 1985; Poole, 1994; Scott *et al*, 1994). It is a minimally invasive procedure, and the appearance of the fluid often gives a good indication of any underlying pathology in the joint e.g. if the fluid is purulent, or contains blood.

In this experiment, the practicalities of obtaining synovial fluid from birds are assessed, and some normal value ranges are established for various characteristics of the fluid. Previous attempts at obtaining synovial fluid from the hip and stifle joints of birds proved unreliable. The hip joint is inaccessible for sampling, and although this is not the case with the stifle, it proved difficult to withdraw much fluid, perhaps because the joint space is quite small, and fat-filled. In contrast, the hock joint is easily accessible, and consistently produced reasonable quantities of fluid. In theory, the hock joint could be accessed with a minimum of trauma in a living bird (Morrow *et al*, 1997), and sampling was therefore restricted to this joint.

This chapter presents the results of the analysis of synovial fluid samples from several different groups of birds. Samples of synovial membrane were also examined histologically, and comparisons made with the results of the fluid analyses.

Background:

The joint cavity in synovial (or diarthrodial) joints consists of a fibrous joint capsule lined by synovial membrane. The synovial tissues are very vascular and rich in lymphatics, while the fibrous capsule and associated ligaments are well-innervated and provide information on proprioception and pain (Banks, 1986). In contrast, the articular cartilage has no blood, nerve or lymphatic supply, and is nourished solely from the synovial fluid (Perman, 1980).

The synovial membrane attaches to the non-weight-bearing part of the articular cartilage, and consists of a lining layer (intima) and a supporting connective tissue layer,

which blends with the fibrous capsule (Banks, 1986). It does not have a basement membrane, being formed by a discontinuous layer of fibroblasts, 1 to 4 cells thick, and macrophages are also present (Perman, 1980). Three types of synovial membrane are described, based on the type of tissues with which they are associated: fibrous, areolar and adipose (Fawthrop *et al*, 1985; Banks, 1986). The surface cells of the synovial membrane are of two main types: type A cells, which contain many vesicles and vacuoles, with prominent Golgi complexes but little rough endoplasmic reticulum (RER), and Type B cells, which have a poorly developed Golgi complex, but well-developed RER. Both of these cell types are thought to have secretory and phagocytic potential (Perman, 1980).

The synovial membrane regulates the volume and macromolecular composition of the synovial fluid within the joint cavity (Perman, 1980). The fluid is a dialysate of blood plasma, with a similar electrolyte composition, but modified by the addition of glycosaminoglycans (mainly hyaluronic acid), secreted by the synovial lining cells (White *et al*, 1978; Perman, 1980, Fawthrop *et al*, 1985). While diffusable substances such as electrolytes can exchange with plasma, larger molecules can leave the synovial space only via the lymphatics (White *et al*, 1978). Changes in synovial fluid composition can result from altered permeability either of the synovial membrane or adjacent tissues (in particular blood vessels), or from disturbances in intra-articular metabolism (Perman, 1980). The main functions of synovial fluid are to provide nutrients for the articular cartilage, and to lubricate the joint.

Information on synovial fluid in birds is limited, the present author having found only one reference which dealt with the cytology of avian synovial fluid (Campbell, 1988). Much has been published on mammalian synovial fluid, however, and the physical characteristics of the fluid seem to be similar between mammals, with minor exceptions (Perman, 1980). Synovial fluid is usually colourless or pale yellow, and should be clear (Fernandez *et al*, 1983), an increase in turbidity suggesting an increased cell count (Houlton, 1994). The volume varies between joints, and can increase in inflammation (but also increases in normal joints following exercise) (Sawyer, 1963; White *et al*, 1978; Fawthrop *et al*, 1985; Houlton, 1994). In mammals, the pH of synovial fluid is usually in the range of 7.3 -7.4 and the specific gravity around 1.010 (White *et al*, 1978).

Synovial fluid is viscous, a very important property which is determined by the concentration and quality (degree of polymerisation) of the hyaluronic acid in the fluid (Perman, 1980; Houlton, 1994). Hyaluronate is added to the plasma dialysate through the activity of type B cells in the synovial membrane, forming polysulphated

glycosaminoglycans (PGAG's), referred to as synovial mucin (Perman, 1980). Synovial fluid is normally quite viscous, although this decreases with inflammation (viscosity often having an inverse relationship with volume), especially in the presence of bacteria which produce hyaluronidase or lysosomal enzymes (Perman, 1980, Fernandez *et al*, 1983).

Protein levels in synovial fluid vary but are normally low (around 1%), however they can increase with inflammation, due to exudation of plasma proteins (White *et al*, 1978; Perman, 1980). Albumin forms 60-70% of the proteins, and fibrinogen is absent. The albumin: globulin ratio also varies, but is normally around 4 (White *et al*, 1978; Perman, 1980). Normal synovial fluid can gel on standing, a property known as 'thixotropism', which can be distinguished from clotting as it regains its fluidity on shaking (Houlton, 1994). Clotting usually occurs secondary to some pathology e.g. if the sample is contaminated by blood, or excessive protein exudation (blood clotting factors and fibrinogen are normally absent from synovial fluid) (Perman, 1980; Fernandez *et al*, 1983; Houlton, 1994).

Various enzymes and electrolytes can also be measured in synovial fluid, but the levels vary, and the changes are often not specific (Houlton, 1994). The following is summarised from Perman (1980): in general, the enzyme levels in synovial fluid are lower than corresponding serum levels (except for alkaline phosphatase, aldolase, chloride and bicarbonate), and correlate with leukocyte numbers. Total inorganic phosphate is equal in both fluids. Glucose is often slightly less in synovial fluid, but can vary widely, and depends on nutritional status. Electrolyte levels are therefore usually compared with a matching serum sample: fasting animals will have levels similar to serum (Houlton, 1994). In bacterial joint infections, monosaccharide levels in the joint fluid are often reduced due to the glycolytic activity of the bacteria, however this is not specific, as large numbers of neutrophils within a joint will also have glycolytic activity e.g. in immune-mediated arthritis (Houlton, 1994). Lipids are absent from synovial fluid (Perman, 1980).

Cytology:

Most of the pathological changes within the joints are related to infection and/or inflammation. The acute inflammatory response has 3 major components: haemodynamic changes, altered vessel permeability and changes in location and concentration of white blood cells (WBC's) (selective migration to the site of inflammation by margination, diapedesis and chemotaxis) (Bainton, 1980). These are reflected in the type of cells present in the synovial fluid.

Inflammation in mammals:

In the early stages, the main cells involved in the acute inflammatory response are neutrophils, which are thought to circulate in blood for about 10 hours, then migrate into the tissues, where they live for 1-2 days. Eosinophils and basophils, present in much smaller numbers, are thought to behave similarly. After a day or two, as the acute reaction subsides, the predominant cells are macrophages (from monocytes). Blood monocytes are thought to circulate in the blood for about 1 day, then become long-lived macrophages in the tissues (for 60-120 days). In more chronic reactions, the cell infiltrate consists of macrophages, lymphocytes, plasma cells and fibroblasts (Bainton, 1980).

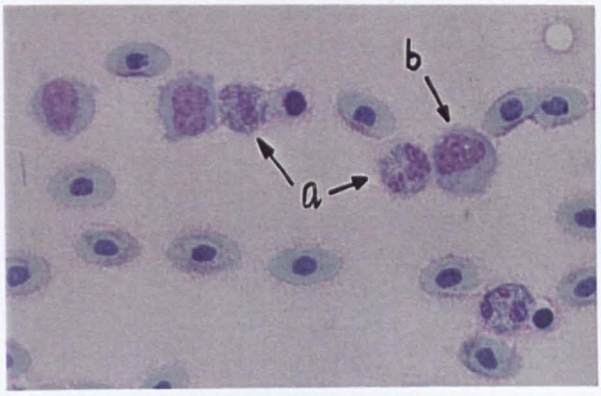
Mammalian synovial fluid cytology:

As a dialysate of plasma, synovial fluid usually contains few cells (Houlton, 1994). Cell counts vary between species, and even between joints (usually related to the presence of inflammation), however leukocyte counts of up to 500 cells per microliter are considered to be normal (Perman, 1980). The cells are usually mainly lymphocytes (around 11-44% of total cells), and other mononuclear cells, which can be derived from cells of synovial lining, tissue macrophages or blood monocytes (Houlton, 1994). Neutrophils are present in low numbers (less than 12% of cell count), usually due to blood contamination (Houlton, 1994). Other cells, such as chondrocytes, osteoblasts and osteoclasts may be seen in synovial fluid, when erosive joint disease has exposed bone (Perman, 1980; Fernandez *et al*, 1983). In general, the body of a normal smear should contain 1-3 nucleated cells per 400x magnification field (Houlton, 1994). (Obviously this will be different in birds, which have nucleated red cells).

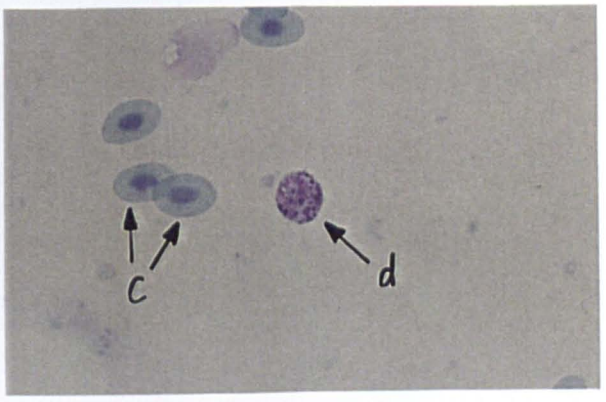
The most clinically significant parameter tends to be the number of polymorphonuclear leucocytes (PMNL's), an influx of neutrophils being necessary for the inflammatory response: normally there are less than 10% PMNL present (but it is also important to consider the absolute numbers) (Perman, 1980). Traumatic arthropathies often have low cell counts with less than 100 mononuclear cells (MN) per microliter. Non-septic polyarthritis usually shows greatly elevated leukocyte counts, and in bacterial joint infections cell counts often exceed 5000MN per microliter (Perman, 1980). The phagocytic activity and degree of vacuolation of the cells is also very important. Those from an infected joint will show toxic changes e.g. pyknosis and degranulation, for example, while those from a case of immune-mediated arthropathy will be more normal (Houlton, 1994).

Avian cytology:

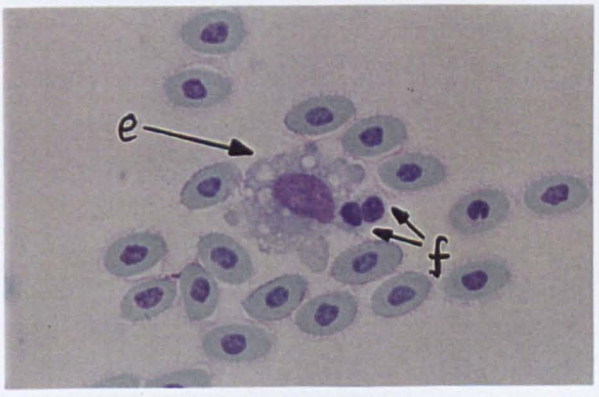
It is useful to start by comparing the major blood cells found in birds and mammals. The details in Table 7.1 are summarised from Lucas and Jamroz (1961), Henrikson and Kaye (1986), Montali, (1988) and Maxwell and Robertson (1995, 1998). Some of the avian cells are illustrated in Figure 7.1 (a-d).



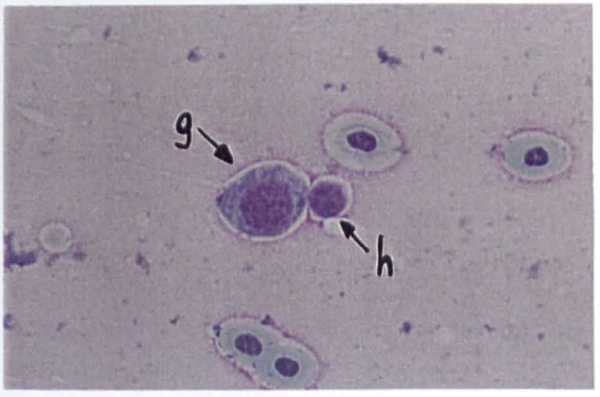
7.1(a) a - heterophils
b - monocyte



7.1(b) c - red blood cells
d - basophil



7.1(c) e – macrophage
f – thrombocytes



7.1(d) g – monocyte
h - lymphocyte

Figure 7.1 (a-d): Some common avian blood cells (photographed under oil immersion, at x 100 magnification).

TABLE 7.1: Comparisons between mammalian and avian blood cells.

MAMMALIAN CELL	'equivalent' AVIAN CELL
Red Blood Cell: *- most numerous blood cell* *- anuclear, size 7-8 μm* *- contains haemoglobin, enabling transfer of gases (O_2 and CO_2).*	**Red Blood Cell:** *- most numerous blood cell, function same as mammalian rbc* *- nucleated cell* *- size 11.4 x 6.8 μm*
Platelet: *- fragments of megakaryocytes* *- important role in haemostasis through aggregation and clotting*	**Thrombocyte:** *- nucleated cell* *- size 6.1 x 11.5 μm long, 3.0 x 6.1 μm wide* *- active phagocyte, not thought to be involved in clotting process*
Neutrophil: *- 60% total WBC's in blood, size 10-12μm* *- multilobed nucleus,* *- blue/purple cytoplasmic granules containing lysosomal enzymes, peroxidase and alkaline phosphatase.* *- acute phase of inflammation* *- phagocytic cells, which function extravascularly*	**Heterophil:** *- 15-25% of total WBC's, average size 8.8μm* *- multilobed nucleus,* *- brick-red cytoplasmic granules containing lysosomal enzymes, but lacking peroxidase and alkaline phosphatase* *- acute phase of inflammation* *- phagocytes, also have role in delayed type hypersensitivity reactions*
Eosinophil: *- 3% total WBC's in blood, size 10-12μm* *- bi-lobed nucleus, bright pink granules* *- increased no's in parasitic infections and allergic reactions.* *-phagocytose Ag-Ab complexes*	**Eosinophil:** *- 0.5-2% of total WBC's, average size 7.9μm* *- bi-lobed nucleus, bright pink granules* *- functions unclear: role in parasitic infections, but not anaphylaxis*
Basophil: *- 0.5% total WBC's in blood, size 10-12μm* *- nuclear morphology often obscured by deep blue granules(containing histamine)* *- 'minor'phagocytic role, also possible role in inflammation and anaphylaxis*	**Basophil:** *- 2-4% total WBC's, average size 9.1μm* *- nuclear morphology often obscured by deep blue granules(containing histamine and heparin)* *- central role in inflammation and hypersensitivity* *- basophilia in severe stress (unique to birds ?)*
Lymphocyte: *- 30% total WBC's in blood, size 7-12μm* *-agranulocyte, minimal cytoplasm* *- important role in immune response via T cells (killer cells) and B cells (plasma cells producing Ab's)*	**Lymphocyte:** *- 50-70% total WBC's,* *- average size 6.1 x 9.2 μm* *- morphology and function similar to mammalian cell*
Monocyte: *- 5% total WBC's in blood, size up to 20μm* *- agranulocyte, variable nuclear morphology, more cytoplasm than lymphocytes* *- form macrophages, with important role in phagocytosis (often vacuolated)*	**Monocyte:** *- 4-5% total WBC's,* *- average size 10.8 x 13.9 μm* *- morphology and function similar to mammalian cell*

Avian inflammation:

Excellent reviews on avian inflammation are presented by Vegad and Katiyar (1995) and Maxwell and Robertson (1998), from which the following summary has been produced.

In the early inflammatory reaction, a heterophil response occurs within about 30 minutes (in both infectious and non-infectious inflammatory reactions). Monocytes and basophils appear within a few hours (basophil degranulation is thought to have a major role in the early phase of acute inflammation). Thrombocytes also appear around this time, and these are very effective phagocytes (more so than either the heterophils or monocytes). Lymphocytes appear later, with lymphoid hyperplasia starting to appear in tissues after about 6 hours, and development of lymphoid aggregates in perivascular tissues variably quoted as taking 36 – 96 hours (see Figure 7.2 d). Although these lymphoid aggregates are very common, their function is unclear, but thought to be analogous to mammalian lymph nodes. After 24 hours, mononuclear cells predominate, particularly macrophages. As the reaction progresses, heterophils can either disappear (by around day 4), or, with macrophage recruitment, develop into characteristic heterophilic granulomas by around day 7.

The stress response has also been studied in detail in birds, and is reviewed by Maxwell (1993). The heterophil / lymphocyte (H/L) ratio is described as a 'good measure of the chicken's perception of the stress in its environment', being less variable than individual cell numbers, and more reliable than corticosterone levels (Gross and Siegel, 1983, quoted by Maxwell, 1993). However, while mild to moderate stress produces a heterophilia and corresponding increase in the H/L ratio, extreme stress has been reported to result in a heteropenia. Severe stress in birds also produces a basophilia (often accompanied by significant heteropenia and lymphocytosis), in contrast to most mammals, where a basopenia occurs (Maxwell, 1993). Interestingly, stress through exposure to continuous high noise produces an increase in monocyte numbers (McFarlane *et al*, 1989, quoted by Maxwell, 1993).

Studies on stress resulting from food restriction produced variable results. An extended period of food withdrawal produced an increased leukocyte count (eosinophilia and monocytosis) (Brake *et al*, 1982, quoted by Maxwell, 1993). Repeated fasts, however, resulted in reduced H/L ratios, suggesting that the birds were becoming habituated (Gross and Siegel, 1986, quoted by Maxwell, 1993). Food restriction of broilers for 4-20 weeks produced no change in H/L ratio, however, but did result in an increase in the numbers of circulating basophils and thrombocytes (Maxwell, 1993).

The aforementioned studies demonstrate the variability of the stress response in birds depending upon the type of stressor. For this reason, the H/L ratio has not been used in the present study, especially as it is difficult to predict how it would be reflected in joint cytology.

Pathological changes:

Many joint diseases produce characteristic changes in the synovial fluid, and a simplified classification (based on mammalian synovial fluid) is presented here, adapted from Banks (1986) and Ettinger (1995):

Parameter	Normal joint	Traumatised joint	Septic joint *	Degenerative joint
volume	'normal'	variable	increased	normal-increased
turbidity	clear	clear-bloody	cloudy	clear
colour	straw	straw-bloody	pink-grey-green	clear or straw
viscosity	high	variable	low	high (*unless excess fluid accumulated*)
mucin clot	good	good	poor	good-fair
fibrin clot	0	0-slight	minimal-marked	0
pH	7 – 7.8	7 – 7.8	decreased	7 – 7.8
total protein (g/dl)	2 – 2.5	variable (increased if serosanguinous)	variable (can be > 4)	2 – 3
RBC's	0	variable	+	0
WBC's (per microliter)	500 - 1000	normal-slightly increased	30,000	1000-5000
cytology	healthy cells	healthy cells (+/- phagocytic vacuoles)	toxic / degenerative cells	healthy cells (+/- phagocytic vacuoles)
monocytes	> 90 %	70 – 80 %	10 – 20 %	> 90 %
neutrophils	<10 %	20 – 30 %	80 – 90 %	< 10 %
SG	1.010	variable	increased	variable

* the nature of the inflammatory exudate depends upon the causal organism (which may be identified in smears).

Examination of synovial fluid is therefore a useful aid in differentiating between various pathological conditions (Perman, 1980; Fernandez *et al*, 1983).

7. 2 MATERIALS AND METHODS

Birds:

Synovial fluid samples were collected from the following groups of birds (which were culled for other reasons):

1) Experimental birds from Chapter 4: these were the relaxed and selected broilers (raised on different feeding regimes) described as Group's A, B, C and D.

2) Several unrelated groups of birds, sampled in a general survey of avian synovial fluid:

Group S1: Twenty normal 6 month old broiler breeders from a commercial poultry unit, which had been raised in floor pens.

Group S2: Ten normal 10 month old Brown Leghorns from Roslin Institute, raised in battery cages.

Group S3: Two 12 week old female broilers that had been injected with mycoplasma at 1 week old, but were not visibly lame at this stage. Raised in floor pens.

Group S4: Twenty normal 18 month old broilers (18 female, 2 male), raised in cages.

Lame group: Fifteen 6 week old broilers, from a commercial poultry unit, raised in floor pens. These birds were selected for culling by the poultry staff, for non-specific lameness problems.

3) Experimental birds from Chapter 6 – groups of selected broilers, raised on either *ad libitum* (A) or restricted (R) feeding regimes, and given either analgesic (C) or placebo (P).

Sample collection:

1. Blood samples: 5mls of blood were taken from the brachial vein (using a 5ml syringe with a 23G x 1" needle), and divided between plain, heparin and oxalate tubes.
2. The birds were killed by an intravenous overdose of anaesthetic (Pentobarbitone sodium, Merial Animal Health).
3. A visual assessment was made of the condition of the hock joints, with each being given a subjective overall score (subsequently meaned between the two joints), based on the 0 – 5 scale used in Chapter 4.
4. Synovial fluid samples were collected from the hock joints. Each joint was cleaned with surgical spirit, and a sterile 21G x 1" needle, attached to a 2 ml syringe, used to aspirate the synovial fluid. The needle was inserted from the caudolateral aspect of

the joint, and the fluid withdrawn under negative pressure.

5. A smear was made of the synovial fluid, and immediately air-dried. The smear was subsequently stained with May-Grunwald-Giemsa (as described in Appendix 7.1) for cytological examination.
6. Assessment of the synovial fluid: the volume of fluid was noted, then a visual assessment made of the colour and turbidity of the fluid, and the presence of old blood or fresh clots noted. A subjective assessment was made of viscosity using the 'string test' (fluid is strung out between finger and thumb, and decreased viscosity noted if fluid strings out to less that 2cm, or drops like water (Fernandez *et al*, 1983; Houlton, 1994)). The remainder of the fluid was then carefully divided between plain, heparin and oxalate tubes, and stored at –20°C.
7. The joints were then opened, and a sample of synovial tissue taken from the anterior aspect of the joint for histological examination. The tissue samples were processed as described in (Appendix 4.3).

Sample processing:

The samples were processed in the laboratory within 24 hours of collection. The plain synovial fluid sample was used for several further tests:
Initially, protein levels were assessed by measuring specific gravity using a refractometer (Houlton, 1994), on the urine specific gravity scale of 1.000-1.050 (Atago Urine Specific Gravity refractometer, Atago, Japan). Synovial fluid pH was then determined using one of two methods. The first set of samples (group S1) were analysed on an automated pH and calcium analyser (634 Ca^{++} / pH analyser, Ciba-Corning). This measured up to pH 8, but surprisingly, many of the sample readings were 'out of range'. Thereafter, samples were measured using pH indicator paper (pH 4.5 – 10, Whatman). Finally, a mucin precipitation test was attempted: 4 parts acetic acid (2%) were added to 1 part synovial fluid, the mixture stirred, and an assessment made of the quality of the clot which formed. A firm clot, in clear solution suggests the synovial fluid hyaluronidase is highly polymerised, which results in good viscosity (Houlton, 1994).

The heparin and oxalate samples (both blood and synovial fluid) were spun at 1500g for 10 minutes (at 5°C), and the supernatant removed by pipette and stored at -70 °C. The plain blood tubes were allowed to stand overnight at room temperature (for maximum clot retraction): they were then spun at 3000g for 10 minutes (at 5°C), the supernatant pipetted off, and stored at -70 °C. It was decided to freeze and store the samples and test

them as a single batch when all the samples had been collected (rather than testing them as separate batches). At a later stage, glucose and protein levels were measured in synovial fluid and serum, using specific kits (Glucose C, Wako and Macro-Protein Assay, Bio-Rad, respectively). As these kits were designed for analysis of plasma samples, it was assumed that they should work equally well on synovial fluid, being a plasma dialysate.

The synovial tissue samples were examined histologically, and an assessment of synovial pathology made, based on the scale used by Morrow *et al* (1997). The thickness of the synovial cell layer (SL) was objectively graded by counting the number of cells, and the degree of synovial and sub-synovial cellular infiltration (CI) was subjectively assessed. The scoring system is described in the table below, and examples of the synovial tissues from each category are illustrated in Figure 7.2 (a-d).

Table 7.2: Scoring method used for assessment of synovial membrane tissue.

score	thickness of synovial cell layer (**SL**)	**score**	degree of synovial and sub-synovial cellular infiltration (including lymphoid whorls) (**CI**)
0	normal (1-3 cells thick)	**0**	normal
1	possible mild change (4-6 cells thick)	**1**	mild infiltrate
2	obvious change (7-11 cells thick)	**2**	obvious infiltrate
3	marked change (> 11 cells thick)	**3**	severe infiltration (with lymphoid whorls).

Manual differential cell counts were performed on the synovial smears, as the presence of nucleated RBC's and thrombocytes prevented the use of automated cell counting equipment. Fifty high power fields (100x magnification) were examined per slide, and the number of cells in each field counted. The cells were grouped into the categories shown in Table 7.3.

Table 7.3: Categories used when counting blood cells.

Gr	Granulocytes (heterophils, eosinophils and basophils)
L	Lymphocytes
Mm	Monocytes and Macrophages
T	Thrombocytes
rbc's	Red blood cells
Gh	Ghost cells i.e. degenerating cells

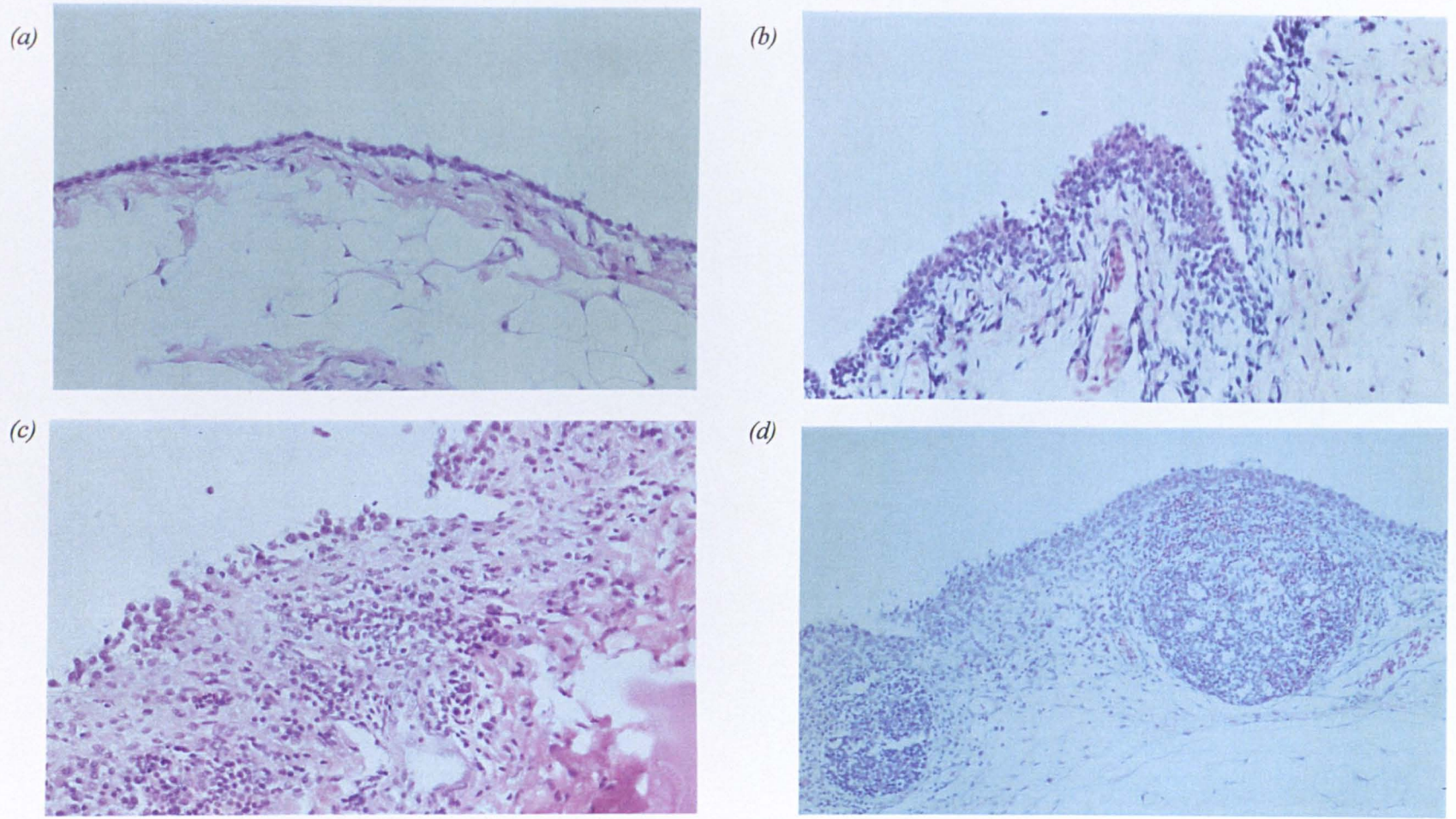


Figure 7.2: Photographs of synovial membrane samples from hock joints of birds in this experiment, to show different grades of synovial layer thickening (SL) and sub-synovial cellular infiltrate (CI). **7.2 (a)** normal synovial layer, no cell infiltrate (both graded 0). **7.2 (b)** mild thickening of synovial layer, with mild cell infiltrate (both graded 1). **7.2 (c)** moderate thickening of the synovial layer (2), with moderate cell infiltrate (2). **7.2 (d)** severe thickening of the synovial layer (3), with obvious lymphoid whorls in sub-synovial layer. *(Figures 7.2 (a-c) x 20 magnification, Figure 7.2(d) x 10 magnification).*

Statistical analysis:

Statistical analysis was confined to the groups from the same controlled experiments. As these birds were 'normal' (and therefore no reason for one leg to be different from the other), the scores for both legs were meaned to simplify the analysis. As the results were counts, and therefore unlikely to be normally distributed, medians are presented, and were compared using Mann-Whitney *U*-tests (non-parametric). The results from the other groups are included for comparison only.

7. 3 RESULTS and DISCUSSION

In general, only very small amounts of fluid (0.1ml) could be obtained from the Brown Leghorn birds (Group S2), limiting the usefulness of this technique in this strain of bird. Although an average of 0.5ml fluid was obtained from each hock of the Group S3 birds, the samples clotted on standing. Samples usually clot due to excess fibrinogen (Perman, 1980, Houlton, 1994), and this may be typical of mycoplasmal infections, or more chronic conditions in general. In contrast, none of the samples from the lame birds clotted (these birds had been lame for only a 'few' days). The results from the other groups are presented in the following tables, in which the following abbreviations are used:

Cl - cell infiltrate (in synovial and sub-synovial layer)
SL - synovial layer thickness (i.e. number of synovial cells).
Gr - granulocytes (heterophils, basophils and eosinophils)
L - lymphocytes
Mm - monocytes and macrophages
T - thrombocytes
rbc - red blood cells
Gh - ghosts
vol - volume (ml)
turb - turbidity
sl.dec. - slightly decreased
p-y - pale yellow
m-y - medium yellow
d-y - deep yellow
bl - blood
cl - fresh clot
o - old blood
o/cl - both old blood and fresh clots present
SG - specific gravity
\+ - slight / mild
++ - obvious
+++ - marked
> - greater than measuring range
c - carprofen
p - placebo

Table 7.4: Analysis of synovial membranes and synovial fluid samples from birds in groups A,B,C and D (from Chapter 4).

	Cl	SL	Gr	L	Mm	T	rbc	Gh
A1L	1.5	2.5	4	4.5	6.5	2.5	807.5	69
A1R	1.5	2	0.5	29	30.5	10.5	281.5	205
A2L	0.5	0.5	1.5	4	5	3	342.5	110
A2R	1	1	3	10.5	12.5	6.5	538	239.5
A3L	1	1	4.5	32	14.5	13.5	562.5	134
A3R	0	0	1	9	3.5	4	507	96.5
A4L	1	2.5	6.5	14.5	17	4.5	937	95
A4R	0.5	1	0	0	0.5	0	11.5	6
A5L	0.5	2	2.5	9.5	6.5	3.5	667.5	141.5
A5R	1	1.5	7.5	8.5	9	0.5	109.5	38.5
A6L	*	*	0	2.5	1.5	7.5	52.5	32.5
A6R	1	2	0	10.5	7	1.5	429	82
A7L	0.5	1.5	26.5	12	25.5	2	679	168.5
GpA median	**1**	**1.5**	**2.5**	**9.5**	**7**	**3.5**	**507**	**96.5**
B1L	1	0.5	0.5	4	2.5	0	31	13
B1R	0.5	0.5	1	2.5	0	1	204.5	96
B2L	0	0	3	12	1.5	5.5	722	109
B2R	0	0	1	4.5	1.5	2.5	173.5	45
B3L	0.5	1	1	10	5	4.5	130.5	67
B3R	0	0	0.5	1.5	1.5	0.5	152	23.5
B4L	1.5	1	0	15	5.5	7.5	505	134
B4R	0	0	2	16	13	6	738	99.5
B5L	0.5	0.5	0	0.5	0	0	4	58
B5R	1	1	0.5	24	7	7	534	93
GpB median	**0.5**	**0.5**	**0.75**	**7.25**	**2**	**3.5**	**189**	**80**
C1L	0	0.5	0	0.5	4.5	0.5	25	12
C1R	0	0	0.5	3	7.5	1.5	192	87.5
C2L	0	0	2.5	5	3.5	0	852.5	217
C2R	0.5	2	0.5	5	17.5	2.5	157	44
C3L	1	1	0.5	1.5	13.5	2	25.5	40
C3R	0	0.5	3.5	2.5	2.5	2	646	256.5
C4L	1	1	0	0	1	0	33	10.5
C4R	0.5	2	4.5	6.5	7	4	405.5	108.5
C5L	1.5	1.5	1	7	11.5	0.5	192	45
C5R	0.5	2	0.5	2	1	0	11	17.5
GpC median	**0.5**	**1**	**0.5**	**2.75**	**5.75**	**1**	**174.5**	**44.5**
D1L	0.5	1	0	6	3.5	1.5	12.5	32
D1R	0	0.5	0	9.5	10.5	0	0.5	36.5
D2L	0	0	0	1	10.5	0	36	39
D2R	0	0	1	3.5	8	2.5	280.5	55
D3L	0.5	0.5	0	3.5	10	0	22	22
D3R	0.5	0	0	2	3	0	5	22
D4L	0	0.5	0	5	6.5	1	8	43
D4R	0	0	0	4	1	3	1	40
D5L	0	0	0	1	5.5	1	5.5	17.5
D5R	0.5	1	0	1	1	0	0.5	15.5
GpD median	**0**	**0.25**	**0**	**3.5**	**6**	**0.5**	**6.75**	**34.25**

* - missing data values

The results in Table 7.4 show that the median scores for cell infiltrate were similar between the groups, except that the GpA birds had significantly higher scores than the Gp D birds ($p<0.002$). The differences in synovial layer thickness between the groups were greater. Gp A had a significantly thicker synovial layer than Gp B ($p<0.004$) and Gp D ($p<0.002$) birds. GpC birds also had a significantly thicker synovial layer than GpD birds ($p<0.05$).

The results of the differential cell counts were more variable between the groups. Gp D birds had significantly lower median granulocyte counts than all the others: GpA ($p<0.002$), and Gp's B and C ($p<0.005$). Gp A birds had significantly greater median lymphocyte counts than Gp C and D birds ($p<0.01$), significantly greater median monocyte / macrophage counts than Gp B birds ($p<0.03$), and significantly greater median thrombocyte counts than both GpC ($p<0.02$) and GP D birds ($p<0.004$).

There was no significant difference in the median red blood cell counts between groups A, B and C. Group D birds had significantly lower median rbc counts than Gp A ($p<0.001$), B and C ($p<0.006$) birds. Group D birds also had the lowest median ghost cell count, significantly lower than both Gp A ($p<0.005$) and B ($p<0.01$) birds.

Table 7.5 (a): Analysis of synovial fluid and synovial membranes from the *ad libitum*-fed birds given carprofen (from Chapter 6).

bird	leg	vol	turb	colour	visc	bl	SG	pH	CI	SL	Gr	L	Mm	T	rbs	Gh
21c	R	0.3	+++	bloody	n	o/cl	1.009	9.0	0	0	0	6	2	1	566	225
	L	0.3	+++	bloody	n	o/cl	1.010	8.5	1	0	0	14	2	1	247	178
30c	R	0.3	+++	bloody	n	o/cl	1.013	8.5	*	*	3	48	5	19	256	49
	L	0.2	+++	bloody	n	o/cl	1.012	8.5	2	2	1	59	11	17	234	72
32c	R	0.4	+++	bloody	n	o/cl	1.010	8.5	0	0	0	22	0	2	214	31
	L	0.5	+++	bloody	sl.dec.	o/cl	1.009	9.0	1	0	0	20	5	8	257	153
38c	R	0.2	+++	m-y	n	cl	1.011	8.0	*	*	*	*	*	*	*	*
	L	0.3	+++	m-y	n	-	1.012	8.0	2	2	*	*	*	*	*	*
31c	R	0.6	+	p-y	n	cl	1.010	8.0	1	1	0	6	35	3	136	58
	L	0.5	+	p-y	n	cl	1.010	8.0	2	1	0	8	100	0	170	254
22c	R	0.5	+++	pink	sl.dec.	o/cl	1.009	8.5	0	1	0	4	16	0	215	383
	L	0.8	+++	bloody	n	o/cl	1.010	8.5	0	1	0	20	11	0	249	19
23c	R	0.3	+++	p-y	n	-	1.012	8.0	1	1	0	15	158	9	23	47
	L	0.4	+++	pink	n	o/cl	1.009	8.0	3	3	0	11	10	2	99	29
26c	R	0.4	+++	p-y	n	-	1.010	8.3	2	2	1	2	2	3	0	27
	L	0.4	+++	m-y	n	cl	1.010	8.3	0	0	1	10	72	3	125	98
%'s and medians		**0.4**	**100% turbid**	**56 % with 'old' blood 81% with clots**			**1.010**	**8.40**	**1**	**1**	**0**	**12.5**	**10.5**	**2.5**	**214.5**	**65**

Table 7.5 (b): Analysis of synovial fluid and synovial membranes from the *ad-libitum*-fed birds given placebo (from Chapter 6).

bird	leg	vol	turb	colour	visc	bl	SG	pH	CI	SL	Gr	L	Mm	T	rbs	Gh
28	R	0.4	+++	bloody	n	o/cl	1.011	8.0	2	2	1	38	78	5	329	162
	L	0.4	+++	m-y	n	c	1.012	8.0	0	1	0	0	248	2	147	59
33	R	0.3	+	p-y	n	-	1.010	8.0	1	1	0	1	2	2	4	4
	L	0.5	+++	m-y	n	-	1.012	8.0	0	0	0	22	5	0	14	8
36	R	0.5	+++	bloody	n	o/cl	1.010	8.0	1	2	1	20	5	2	210	19
	L	0.4	+++	bloody	n	o/cl	1.010	8.3	1	0	0	16	0	25	97	247
27	R	0.3	+++	bloody	n	o/cl	1.012	8.3	3	2	0	6	2	1	293	97
	L	0.4	+++	bloody	n	o	1.012	8.3	1	0	0	7	10	8	109	23
40	R	0.4	+++	bloody	n	o/cl	1.010	8.5	0	1	0	2	15	2	128	44
	L	0.3	+++	bloody	sl.dec.	o/cl	1.009	8.5	1	2	3	53	8	41	755	293
39	R	0.2	+++	m-y	sl.inc.	-	1.011	8.0	1	2	0	22	2	10	12	26
	L	0.4	+++	m-y	n	o	1.011	8.0	2	1	0	12	1	1	1	17
37	R	0.3	blood	bloody	n	o/cl	1.008	8.3	2	2	0	18	11	6	281	114
	L	0.4	+++	bloody	n	o	1.009	8.3	1	1	*	*	*	*	*	*
35	R	0.3	+++	bloody	n	o/cl	1.009	8.5	0	1	0	44	91	12	84	49
	L	0.6	+	p-y	n	-	1.012	8.0	1	1	0	14	96	4	3	35
%'s and medians		**0.4**	**100% turbid**	**62% with 'old' blood 56% with clots**			**1.011**	**8.15**	**1**	**1**	**0**	**16**	**8**	**4**	**109**	**44**

Table 7.6 (a): Analysis of synovial fluid and synovial membranes from the restricted-fed birds given carprofen (from Chapter 6).

bird	leg	vol	turb	colour	visc	bl	SG	pH	Cl	SL	Gr	L	Mm	T	rbs	Gh
18c	R	0.4	+	p-y	n	-	1.010	8.0	1	0	0	9	0	0	1	32
	L	0.6	+++	p-y	n	-	1.009	7.5	0	0	0	0	0	0	0	0
1c	R	0.4	+	p-y	n	-	1.007	7.5	1	0	0	0	0	0	0	11
	L	0.6	+	m-y	n	-	1.008	7.5	0	1	0	1	0	0	0	28
13c	R	0.6	+++	m-y	n	o/cl	1.008	8.5	0	0	1	5	1	0	0	118
	L	0.7	+	p-y	n	-	1.008	8.0	0	1	0	0	0	0	0	2
10c	R	0.6	+	p-y	n	-	1.008	7.5	0	0	0	3	6	0	3	9
	L	0.6	+	p-y	n	-	1.008	8.0	0	1	0	2	0	0	0	11
14c	R	0.3	+	p-y	n	cl	1.008	7.5	2	1	0	1	0	0	0	2
	L	0.4	+	p-y	n	-	1.010	7.5	1	0	0	1	2	0	38	74
15c	R	0.2	+	p-y	n	-	1.009	7.5	0	0	0	1	0	0	0	5
	L	0.2	+	p-y	n	-	1.009	7.5	0	0	0	4	0	0	0	5
16c	R	0.5	clear	clear	n	-	1.007	7.5	*	*	0	0	0	0	0	1
	L	0.6	+	p-y	n	-	1.007	7.5	0	0	0	0	0	0	0	0
4c	R	0.5	+++	m-y	n	o/cl	1.007	7.5	0	0	0	0	0	0	0	42
	L	0.6	+++	m-y	n	-	1.008	7.5	*	*	0	2	0	0	0	32
7c	R	0.6	+++	p-y	n	c	1.010	7.0	1	1	0	2	0	0	0	40
	L	0.6	clear	clear	n	-	1.007	7.0	2	2	0	0	0	0	0	11
%'s and medians		**0.6**	**88.9% turbid**	**11% with 'old' blood, 22.2% with clots**			**1.008**	**7.5**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**11**

Table 7.6 (b): Analysis of synovial fluid and synovial membranes from the restricted-fed birds given placebo (from Chapter 6).

bird	leg	vol	turb	colour	visc	bl	SG	pH	CI	SL	Gr	L	Mm	T	rbs	Gh
8	R	0.8	+	p-y	n	-	1.008	8.0	1	2	0	5	0	0	2	62
	L	0.9	clear	p-y	sl.dec.	-	1.007	8.0	1	1	0	0	0	0	0	0
6	R	0.7	+	p-y	n	cl	1.007	7.5	1	1	0	2	0	0	0	2
	L	0.5	+	p-y	n	+	1.009	7.5	*	*	0	1	0	0	0	9
20	R	0.5	+	p-y	n	-	1.009	7.0	*	*	0	3	1	0	3	12
	L	0.5	clear	clear	n	-	1.008	8.0	1	1	0	1	3	0	0	17
11	R	0.7	+	p-y	n	-	1.007	7.5	0	1	0	1	0	0	0	14
	L	0.8	+++	pink	n	o	1.009	7.5	2	2	0	20	0	0	96	126
17	R	0.6	+	p-y	n	cl	1.007	7.0	*	*	0	0	0	0	0	29
	L	0.8	clear	clear	n	-	1.007	7.0	*	*	0	2	0	0	0	15
5	R	0.8	+++	m-y	sl.dec.	-	1.008	7.5	1	1	0	3	1	0	1	96
	L	0.8	blood	bloody	n	o	1.009	8.0	0	1	0	2	0	0	0	213
12	R	0.3	+	p-y	n	-	1.010	8.0	0	0	0	6	0	0	3	11
	L	0.3	+++	m-y	n	cl	1.010	8.0	2	1	0	6	2	0	12	102
19	R	0.5	+	p-y	n	-	1.009	8.0	1	1	0	3	0	0	0	39
	L	0.4	+	p-y	n	cl	1.008	7.5	0	1	0	4	1	0	0	160
%'s and medians		**0.65**	**81.2% turbid**	**12% with 'old' blood, 31 % with clots**			**1.008**	**7.5**	**1**	**1**	**0**	**2.5**	**0**	**0**	**0**	**23**

Tables 7.5 (a, b) and 7.6 (a, b) show the results of analysis of the synovial fluid samples from birds in the experiment described in Chapter 6. Larger median volumes of synovial fluid were obtained from the restricted-fed birds than from those fed *ad libitum*. The median volume from the restricted-fed birds given placebo was significantly greater than that from the *ad libitum*-fed birds given placebo ($p<0.01$) or carprofen ($p<0.03$), and the restricted-fed birds given carprofen had significantly greater volumes than the *ad libitum*-fed birds given placebo ($p<0.0326$). The samples from the *ad libitum*-fed birds were generally more turbid, and also contained more blood (both old blood and fresh clots), reflecting the higher cell counts in these birds.

The samples from the *ad libitum*-fed birds had greater median SG's than those from the restricted-fed birds, the significance varying depending upon the groups being compared, but ranging from $p<0.004$-0.001. Likewise, the median pH of the synovial fluid was significantly greater in the *ad libitum*-fed birds compared to that of the restricted-fed birds, the significance again depending upon the groups being compared, but ranging from $p<0.001$-0.002.

The values for cell infiltrate and synovial membrane thickness were similar between the groups, with a few exceptions. The cell infiltrate for the restricted-fed birds given carprofen was significantly lower than that for the *ad libitum*-fed birds, given either carprofen ($p<0.05$) or placebo ($p<0.04$). The synovial membrane thickness was also significantly lower in the restricted-fed birds given carprofen than either the restricted-fed birds given placebo ($p<0.01$), or the *ad libitum*-fed birds given placebo ($p<0.006$).

The results of the differential cell counts showed no significant difference in granulocyte and thrombocyte numbers between the groups. The *ad libitum*-fed birds had significantly greater numbers of lymphocytes and monocytes/macrophages than the samples from the restricted-fed birds, with no apparent treatment effect (significance varying upon the comparison being made, $p< 0.005$-0.0006 for lymphocytes, and $p<0.001$-0.002 for monocyte/macrophage numbers). Similarly for rbc numbers, the *ad libitum*-fed birds had significantly greater numbers of rbc's in their samples than the restricted-fed birds, irrespective of treatment (range $p< 0.003$-0.0008). The results of the ghost cell counts were more variable, with the restricted-fed birds given carprofen having significantly fewer ghost cells than *ad libitum*-fed birds given either carprofen ($p<0.002$) or placebo ($p<0.003$).

Table 7.7: Analysis of synovial fluid and synovial membranes from the birds in the S1 Group

bird	leg	score	vol	turb	colour	bl	SG	pH	CI	SL	Gr	L	Mm	T	rbs	Gh
1	R	2	0.5	+	p-y	cl	1.016	>	2	1	0	7	4	7	31	99
	L	3	0.6	clear	clear	-	1.013	7.93	1	0	0	0	0	1	1	16
2	R	2	0.3	+	p-y	-	1.015	>	0	1	0	0	0	3	11	25
	L	0	0.3	++	p-y	-	1.015	>	0	1	0	0	2	2	17	48
3	R	0	0.6	bloody	bloody	o/cl	1.014	7.82	1	0	0	8	18	0	59	181
	L	2	0.5	++	m-y	-	1.014	>	0	1	0	1	7	0	0	12
4	R	1	0.7	+	p-y	cl	1.015	7.90	1	1	0	3	0	37	2	16
	L	1	0.9	clear	p-y	-	1.013	7.92	*	*	*	*	*	*	*	*
5	R	2	0.5	+	p-y	cl	1.014	>	0	0	0	3	1	10	7	35
	L	2	0.5	+	p-y	cl	1.013	*	0	0	0	1	1	4	1	21
6	R	0	0.5	clear	p-y	cl	1.014	>	0	1	0	2	1	8	3	82
	L	0	0.6	++	d-y	-	1.014	>	0	0	0	2	0	4	3	38
7	R	0	0.7	bloody	bloody	o/cl	1.017	>	0	0	0	3	3	6	63	94
	L	0	0.5	++	d-y	cl	1.014	*	1	2	0	4	7	30	51	191
8	R	1	0.4	clear	clear	cl	1.014	>	0	0	0	1	2	3	3	67
	L	1	0.3	clear	clear	cl	1.016	7.87	0	0	0	6	1	5	276	873
9	R	2	0.3	clear	clear	-	1.014	>	1	1	0	4	1	11	11	51
	L	1	0.4	clear	clear	-	1.014	>	0	0	0	0	1	1	0	17
10	R	1	0.4	+	p-y	-	1.015	*	*	*	0	1	2	6	2	27
	L	1	0.6	clear	p-y	-	1.015	>	0	0	0	4	0	13	1	20

Table 7.7 (cont): Analysis of synovial fluid and synovial membranes from the birds in the S1 Group

bird	leg	score	vol	turb	colour	bl	SG	pH	CI	SL	Gr	L	Mm	T	rbs	Gh
11	R	1	0.3	clear	clear	-	1.013	>	1	1	0	2	0	6	9	19
	L	1	0.4	+	clear	-	1.014	>	2	2	0	0	2	4	4	40
12	R	1	0.5	+	p-y	-	1.013	>	0	1	0	1	1	0	0	7
	L	0	0.6	+	p-y	-	1.013	>	0	2	0	2	0	2	15	48
13	R	2	0.5	+	p-y	cl	1.014	>	1	2	0	6	0	3	2	45
	L	2	0.4	+	p-y	-	1.015	>	0	1	0	2	0	5	12	33
14	R	2	0.7	+	p-y	-	1.014	*	1	1	0	0	1	0	0	2
	L	2	0.6	+	p-y	-	1.015	8.0	1	1	2	8	3	7	105	85
15	R	1	0.4	+	p-y	-	1.014	>	0	1	0	1	0	0	14	49
	L	3	0.5	+	p-y	-	1.015	>	0	1	0	1	2	1	0	23
16	R	0	0.3	clear	clear	cl	1.014	>	0	2	0	1	0	1	130	208
	L	1	0.4	+	p-y	-	1.015	>	0	2	0	0	2	1	5	23
17	R	2	0.5	+	d-y	-	1.014	>	1	0	0	0	0	0	2	11
	L	3	0.5	clear	clear	-	1.013	>	0	1	0	1	0	3	44	102
18	R	2	0.3	+	p-y	cl	1.017	7.97	1	1	0	9	7	0	33	63
	L	2	0.3	+++	d-y	o	1.018	7.64	2	2	0	3	2	0	14	37
19	R	0	0.4	clear	clear	-	1.013	7.81	0	0	0	0	0	0	0	9
	L	0	0.5	clear	clear	-	1.012	7.79	1	1	0	3	1	3	2	21
20	R	2	0.5	clear	clear	cl	1.016	7.78	0	1	0	0	0	0	4	5
	L	2	0.4	clear	p-y	cl	1.016	7.71	1	1	0	1	2	5	12	95
median		**0**	**0.5**				**1.014**	**7.84**	**0**	**1**	**0**	**1**	**1**	**3**	**5**	**37**

Table 7.8: Analysis of synovial fluid and synovial membranes from the birds in the S4 Group

bird	leg	score	vol	turb	colour	bl	SG	pH	Cl	SL	Gr	L	Mm	T	rbs	Gh
1	L	0	0.6	+	p-y	-	1.014	8.5	0	0	0	2	0	0	17	10
	R	0	0.6	+	p-y	-	1.016	8.5	0	0	0	0	2	0	2	1
2	L	0	0.4	+	p-y	-	1.014	8.0	0	0	0	1	2	0	8	8
	R	0	0.4	++	p-y	cl	1.014	8.0	0	1	0	0	0	0	1	0
3	L	1	0.6	+	clear	-	1.012	8.0	0	1	0	1	3	0	93	13
	R	1	0.7	clear	clear	-	1.013	7.5	0	0	0	0	1	0	0	4
4	L	0	0.4	clear	clear	cl	1.016	8.0	0	0	0	1	1	1	70	11
	R	0	0.4	clear	clear	-	1.016	7.5	0	0	0	1	2	1	24	7
5	L	0	0.4	clear	clear	-	1.014	8.0	0	0	0	5	4	0	2	4
	R	0	0.5	clear	clear	-	1.014	8.0	0	0	0	0	6	0	0	2
6	L	0	0.3	clear	clear	-	1.015	8.0	0	1	0	3	2	0	81	42
	R	0	0.3	clear	clear	-	1.016	8.0	0	1	0	3	2	0	2	9
7	L	0	0.3	+	p-y	-	1.017	8.0	0	2	0	18	25	2	12	19
	R	0	0.2	+	p-y	-	1.014	8.5	1	2	0	7	14	1	23	23
8	L	1	0.5	+	p-y	-	1.015	8.0	0	2	0	0	0	0	0	0
	R	0	0.5	+	clear	-	1.014	8.0	0	1	0	0	1	0	1	1
9	L	1	0.7	+	clear	-	1.015	8.0	0	0	0	4	2	0	0	2
	R	0	0.5	+	clear	-	1.016	8.0	1	0	0	0	1	0	2	0
10	L	0	0.4	+	p-y	-	1.015	8.0	0	1	0	0	0	0	78	32
	R	0	0.6	+	clear	-	1.013	8.0	0	0	0	0	1	0	0	12

Table 7.8 (cont): Analysis of synovial fluid and synovial membranes from the birds in the S4 Group

bird	leg	score	vol	turb	colour	bl	SG	pH	Cl	SL	Gr	L	Mm	T	rbs	Gh
11	L	0	0.4	clear	clear	-	1.014	8.0	0	1	0	0	8	0	47	20
	R	0	0.3	clear	p-y	-	1.015	8.0	1	1	0	2	8	0	16	17
12	L	0	0.3	clear	clear	-	1.015	8.0	0	0	0	1	1	0	14	19
	R	0	0.3	+	clear	-	1.014	8.5	0	1	0	1	4	0	42	26
13	L	0	0.3	clear	clear	cl	1.016	8.0	1	2	0	4	5	7	330	38
	R	0	0.3	+	p-y	cl	1.016	9.0	0	2	0	1	2	0	43	15
14	L	1	0.5	clear	clear	-	1.014	8.0	0	0	0	0	2	0	3	5
	R	2	0.5	clear	clear	-	1.013	8.5	0	0	0	0	0	0	0	5
15	L	0	0.3	+	m-y	-	1.014	8.5	1	2	0	0	4	1	22	12
	R	0	0.2	+	m-y	-	1.013	8.5	2	3	0	3	0	0	0	12
16	L	0	0.7	+	m-y	-	1.013	8.5	1	2	*	*	*	*	*	*
	R	0	0.4	+	clear	-	1.013	8.5	0	1	*	*	*	*	*	*
17	L	0	0.3	+	p-y	cl	1.014	8.5	0	0	0	2	1	0	4	5
	R	0	0.4	clear	p-y	cl	1.016	8.5	0	1	0	0	0	0	1	0
18	L	0	0.3	clear	clear	cl	1.013	8.0	0	1	0	0	1	1	10	4
	R	0	0.3	+	m-y	-	1.014	8.5	1	1	0	1	5	1	24	12
19	L	0	0.3	clear	clear	-	1.014	8.5	1	1	0	1	1	0	0	0
	R	0	0.3	clear	clear	-	1.015	8.5	0	0	0	2	6	0	0	6
20	L	1	0.8	+	clear	-	1.015	8.5	1	2	0	0	1	0	0	15
	R	1	0.5	+	p-y	-	1.016	8.0	0	1	0	0	2	0	3	4
median		**0**	**0.4**				**1.014**	**8.0**	**0**	**1**	**0**	**1**	**2**	**0**	**3.5**	**8.5**

Table 7.9: Analysis of synovial fluid and synovial membranes from the birds in the Lame Group

bird	leg	score	vol	turb	colour	bl	SG	pH	Cl	SL	Gr	L	Mm	T	rbs	Gh
1	L	1	0.3	+	p-y	-	1.016	8.5	1	2	101	61	57	6	442	111
	R	4	0.9	+++	grey	-	1.022	8.5	0	1	112	1	6	0	5	27
2	L	1	0.2	clear	p-y	-	1.015	8.5	0	0	3	6	4	0	532	142
	R	1	0.2	+	p-y	-	1.015	8.5	0	1	1	20	10	6	221	100
3	L	1	0.3	+++	grey	-	1.032	8.5	0	0	268	15	48	1	300	123
	R	2	0.5	+++	p-y	-	1.030	8.0	0	1	227	25	51	2	416	71
4	L	1	0.5	+++	m-y	-	1.040	7.0	0	1	512	7	44	0	6	21
	R	1	0.4	+++	m-y	-	1.038	7.5	0	0	79	69	105	0	12	74
5	L	2	0.5	+++	p-y	-	1.024	8.0	*	*	686	11	68	1	1	45
	R	3	0.6	+++	p-y	-	1.028	8.0	1	1	435	41	30	2	298	169
6	L	3	0.4	+++	grey	-	1.036	7.5	1	1	448	15	95	0	75	39
	R	2	0.2	+++	grey	-	1.033	8.3	1	1	414	4	26	0	21	27
7	L	1	0.3	+++	p-y	-	1.032	8.0	1	2	477	15	50	1	1	28
	R	1	0.5	+++	p-y	-	1.033	8.3	1	2	368	5	23	0	2	15
8	L	0	0.3	clear	p-y	-	1.016	8.5	0	1	1	1	4	1	69	118
	R	1	0.4	clear	clear	-	1.016	8.5	1	2	1	6	16	0	82	50
9	L	1	0.8	+++	grey	-	1.025	8.0	1	1	294	12	28	0	33	41
	R	1	0.5	+++	grey	-	1.026	8.5	0	0	48	1	2	0	0	2
10	L	0	0.3	+++	bloody	o/cl	1.018	8.5	0	1	33	12	15	13	187	145
	R	0	0.3	clear	p-y	-	1.020	8.5	0	0	0	1	10	0	12	6

Table 7.9 (cont): Analysis of synovial fluid and synovial membranes from the birds in the Lame Group

bird	leg	score	vol	turb	colour	bl	SG	pH	CI	SL	Gr	L	Mm	T	rbs	Gh
11	L	2	0.9	+++	grey	-	1.024	8.0	1	2	310	21	18	0	0	11
	R	2	0.8	+++	green	-	1.024	8.0	0	1	764	32	58	2	4	49
12	L	2	0.4	+++	bloody	o/cl	1.031	7.5	0	2	242	4	15	0	31	26
	R	2	0.9	+++	m-y	o/cl	1.026	8.3	1	3	588	29	27	3	233	123
13	L	1	0.2	+	p-y	o/cl	1.022	8.5	1	2	11	2	6	0	103	35
	R	1	0.2	+++	p-y	-	1.025	8.5	*	*	75	10	58	0	77	27
14	L	1	0.2	+++	p-y	-	1.023	8.5	1	2	340	29	75	4	427	33
	R	2	0.3	+++	p-y	-	1.026	8.5	*	*	177	0	13	0	0	177
15	L	2	0.7	+++	green	-	1.035	7.5	2	3	316	1	40	0	0	19
	R	1	0.1	+	p-y	-	1.032	8.5	0	0	3	0	2	0	36	16
16	L	1	0.5	+++	p-y	-	1.032	8.0	*	*	103	48	130	4	13	30
	R	2	0.3	+++	p-y	-	1.032	8.0	*	*	575	18	55	0	7	4
17	L	2	0.6	+++	p-y	-	1.028	8.5	*	*	81	1	10	0	0	18
	R	2	0.3	+++	m-y	o/cl	1.028	7.5	*	*	169	2	18	0	20	20
18	L	0	0.2	clear	clear	-	1.032	8.5	*	*	1	9	64	2	5	12
	R	3	0.5	+++	p-y	-	1.032	8.3	*	*	142	11	14	0	0	37
19	L	1	0.2	+	bloody	o/cl	1.019	8.5	*	*	*	*	*	*	*	*
	R	3	0.3	+++	bloody	o/cl	1.022	8.3	*	*	*	*	*	*	*	*
median		**1**	**0.35**				**1.026**	**8.3**	**0**	**1**	**173**	**10.5**	**26.5**	**0**	**20.5**	**31.5**

TABLE 7.10: Summary table: medians (and ranges) of results of analyses of synovial membrane and synovial fluid samples.

	history	CI	SL	Gr	L	Mm	T	rbc	Gh
Gp A: *ad libitum*-fed selected broilers	6 weeks old, raised in floor pens	**1** (0-1.5)	**1.5** (0-2.5)	**2.5** (0-26.5)	**9.5** (0-32)	**7** (0.5-30.5)	**3.5** (0-13.5)	**507** (11-937)	**96.5** (6-239)
Gp B: *ad libitum*-fed relaxed broilers	12 weeks old, raised in floor pens	**0.5** (0-1.5)	**0.5** (0-1)	**0.75** (0-3)	**7.25** (0.5-24)	**2** (0-13)	**3.5** (0-7.5)	**189** (4-738)	**80** (13-134)
Gp C: restricted-fed selected broilers	13 weeks old, raised in floor pens	**0.5** (0-1.5)	**1** (0-2)	**0.5** (0-4.5)	**2.75** (0-7)	**5.75** (1-17.5)	**1** (0-4)	**174.5** (11- 852)	**44.5** (10-256)
Gp D: restricted-fed relaxed broilers	24 weeks old, raised in floor pens	**0** (0-0.5)	**0.25** (0-1)	**0** (0-1)	**3.5** (1-9.5)	**6** (1-10.5)	**0.5** (0-3)	**6.7** (0.5-280)	**34.2** (15-55)
S1 group restricted-fed broiler breeders	commercial birds, 24 weeks old, raised in floor pens	**0** (0-2)	**1** (0-2)	**0** (0-2)	**1** (0-9)	**1** (0-18)	**3** (0-37)	**5** (0-276)	**37** (2-873)
S4 group *ad libitum*-fed selected broilers	72 weeks old, raised in cages	**0** (0-2)	**1** (0-3)	**0** (0)	**1** (0-18)	**2** (0-25)	**0** (0-7)	**3.5** (0-330)	**8.5** (0-42)
Lame group *ad libitum*-fed selected broilers	commercial birds, 6 weeks old, raised in floor pens	**0** (0-2)	**1** (0-3)	**173** (0-764)	**10.5** (0-69)	**26.5** (2-130)	**0** (0-13)	**20.5** (0-532)	**31.5** (2-169)
ad libitum-fed selected broilers given carprofen	6 weeks old, raised in floor pens	**1** (0-3)	**1** (0-3)	**0** (0-3)	**12.5** (2-59)	**10.5** (0-158)	**2.5** (0-19)	**214.5** (0-566)	**65** (19-383)
ad libitum-fed selected broilers given placebo	6 weeks old, raised in floor pens	**1** (0-3)	**1** (0-2)	**0** (0-3)	**16** (0-53)	**8** (0-248)	**4** (0-41)	**109** (1-755)	**44** (4-293)
restricted-fed selected broilers given carprofen	13 weeks old, raised in floor pens	**0** (0-2)	**0** (0-2)	**0** (0-1)	**1** (0-9)	**0** (0-6)	**0** (0)	**0** (0-38)	**11** (0-118)
restricted-fed selected broilers given placebo	13 weeks old, raised in floor pens	**1** (0-2)	**1** (0-2)	**0** (0)	**2.5** (0-20)	**0** (0-3)	**0** (0)	**0** (0-96)	**23** (0-213)

The results from the three groups of 'survey' samples are presented in Tables 7.7, 7.8 and 7.9. The results presented in Tables 7.7 and 7.8 show few differences in the synovial membrane scores (CI and SL) and cell counts from those of *ad libitum*-fed birds presented in the previous tables. One marked difference is the much higher numbers of rbc's and ghost cells found in the samples of the *ad libitum*-fed birds from experiment 7 (Tables 7.5a, 7.5b.).

The results of the analysis of synovial fluid from the group of lame birds are presented in Table 7.9. These samples showed a larger range of colours, and a higher median SG than most of the other sets of samples from normal *ad libitum*–fed broilers. The most dramatic feature of this set of samples was the presence of large numbers of granulocytes, in contrast to their rarity in all the previous samples. Surprisingly, however, the scores for the synovial membrane thickness and cell infiltrate were similar to those of the normal birds, and there were fewer rbc's and ghosts present than in the samples from the experimental birds (Tables 7.4, 7.5a, 7.5b, 7.6a, 7.6b). The numbers of other cell types were variable: similar numbers of lymphocytes to the other groups, slightly more monocytes and macrophages.

The results demonstrate that synovial fluid samples are easily obtained from the hock joints of broilers, in sufficient quantities to run several useful tests. Although this was not the case with the Brown Leghorns (being smaller birds), larger birds such as turkeys should be even easier to sample.

Histology / cytology: Histological examination of the synovial membrane was simple, however the information that it provides is limited, and occasionally there was insufficient synovial membrane in the sample to make a clear assessment. Although there was little difference in the degree of cellular infiltrate between the various groups, significant differences were found in the thickness of the synovial cell layer. In the birds from Chapter 4, it was particularly notable that the *ad libitum*-fed selected broilers had significantly thicker synovial cell layers than the relaxed birds.

Differential cell counts are much more time consuming (particularly if rbc's and ghost cells are counted), but provide lots of potentially useful information. Care has to be taken in interpreting the significance of the leucocytes present in samples where blood contamination through needle trauma has occurred however. It is therefore important to consider a particular cell count with reference to the percentage of the total WBC count that

cell type would be expected to make up in a normal blood smear. The main difficulties in identifying the various cell types were in differentiating between small lymphocytes and thrombocytes. It was also difficult to distinguish between heterophils and eosinophils, however in the cell counts they were both counted as granulocytes.

It was interesting that the feeding regime appeared to have a more significant effect on the differential cell counts than the drug treatments in the experimental birds described in Chapter 6. The *ad libitum*-fed birds in that experiment had significantly greater numbers of lymphocytes, monocytes/macrophages and rbc's in their samples than the restricted-fed birds, irrespective of whether they received carprofen or placebo. It is particularly notable that the *ad libitum*-fed birds from Chapter 6 had far greater numbers of rbc's and ghosts than the *ad libitum*-fed birds in the S1, S4 and lame groups. It is interesting to speculate as to whether this may have arisen because the experimental birds described in Chapter 6 were encouraged to walk along a runway for the gait analysis. Although the exercise was relatively mild (approximately 10 runs, on two occasions, 5 days apart), it may have been sufficiently stressful in these rapidly growing, heavy birds, to traumatise the joints. Birds reared in commercial situations may choose to be less active.

A second interesting finding was the rarity of granulocytes in all the samples, except for the samples from the lame birds where they were abundant. These granulocytes appeared to be predominantly heterophils (estimated at greater than 95%). As discussed in the introduction, heterophils are the earliest white blood cells to appear in the inflammatory response, and usually start to disappear after around 4 days (Maxwell and Robertson, 1998). As these birds were reared on a commercial farm, it would be hoped that they would indeed have been lame for no more than a few days. The heterophil is the avian 'equivalent' of the mammalian neutrophil, and neutrophil numbers are known to increase dramatically in septic joint infections in mammals. Increased numbers of mononuclear cells (lymphocytes, monocytes and macrophages) were also present, but the increase was far less dramatic. It was perhaps surprising not to find a greater number of phagocytic mononuclear cells however, particularly thrombocytes and macrophages, if the joints were infected (as suggested by the heat, inflammation, degree of lameness and colour of many of the synovial fluid samples). It is possible that the leucocytic response to infection / inflammation within joints may differ from that in peripheral blood. Serial sampling of joint fluid samples from birds with joint infections created in a controlled experiment, would be required to investigate this further.

Joint score: The joint scores of the birds raised in cages (S4) were lower than those raised in floor pens (S1). This is not surprising, as the hocks of birds raised in pens often become reddened due to the birds sitting in the deep-litter, particularly if the litter is moist. The lame birds' scores were highest of all.

Volume: The average volume of fluid obtained from the birds' joints was similar between each group, although the quantities from individual joints varied considerably. Although an inflamed joint will normally contain more synovial fluid (White *et al*, 1978; Fawthrop *et al*, 1985; Lumsden *et al*, 1996), other factors can influence the quantity which is obtained e.g. high fibrinogen levels could cause it to clot, or inflamed synovial membrane may block the needle (Houlton, 1994). This could explain why smaller volumes were obtained from the *ad libitum*–fed birds than the restricted-fed birds (the samples from the former being more turbid, and having higher median SG's). The joint spaces in birds are quite small, making sampling difficult, and so the experience of the sampler also plays a significant role. Overall, volumes from birds in the 'normal' groups ranged from 0.2 - 0.9mls, demonstrating that quite large amounts can be obtained relative to other species of greater size. Other studies have reported ranges of 0.1-1ml from the larger joints in normal dogs (Houlton, 1994), 0.2-2mls from normal joints of humans (Fawthrop *et al*, 1985), 4-20 mls from tibiotarsal joints in cattle (Perman, 1980, quoting Van Pelt and Conner, 1963a) and 3.3-10.3 mls from various joints in horses (Perman, 1980, quoting Van Pelt, 1962).

Viscosity: Viscosity was quite difficult to assess, and is very subjective. The samples from the S1 and S4 groups seemed to be of normal viscosity, or slightly increased. In the lame birds, 63.2% were normal, 5.2% slightly increased, but 31.6% seemed to be more watery. This is not surprising, as viscosity can be decreased if the synovial fluid is diluted by effusion, or bacteria are present which produce hyaluronidase, which degrades the mucin (Perman, 1980; Fernandez *et al*, 1983; Fawthrop *et al*, 1985).

Turbidity: There were obvious differences in the turbidity of the samples from the various groups. Of the S1 and S4 birds, 82.5-95 % of the samples were either clear, or only slightly cloudy. In contrast, only 26.4% of the lame birds' samples were clear or slightly cloudy, and the remainder were very cloudy. This was indicative of pathology within the joint, as increased turbidity usually results from increased numbers of cells, either through infection or inflammation (Fawthrop *et al*, 1985; Houlton, 1994). It was therefore surprising to find that most of the samples from the apparently normal broilers from Chapter 6 were also very

turbid, suggesting a sub-clinical pathological process occurring within these birds joints (e.g. chronic low-grade inflammation).

Colour: The colour of the synovial fluid samples varied widely. Most of the S1 and S4 birds had normal clear or pale yellow joint fluid, however 12.5-15% of the samples were medium yellow, suggesting the presence of old blood (breakdown products). Recent unclotted haemorrhage tends to appear pink. In contrast, only half of the samples from the birds in the lame group had normal coloured fluid, with 26% greyish/green, and 10 % bloody; the colour can depend on the presence of bacteria within the joint (Perman, 1980). A surprisingly large number of *ad libitum*-fed birds from the experiment described in Chapter 6 had bloody synovial fluid.

Blood: Fresh blood in the synovial fluid is obvious (as a clot), and usually occurs due to needle trauma during sampling. This can be confirmed in mammals by examining a smear for the presence of platelets, as platelets are usually absent from normal or disease-related samples (Perman, 1980; Fernandez *et al*, 1983). This is not appropriate in birds, however, as they do not have platelets. Thrombocytes are primarily active phagocytes, which were originally thought not to have a role in clotting (Gross, 1989, quoted by Maxwell, 1993), however recent work suggests this may not be the case (Maxwell, pers.com.). Fresh blood was quite common in the samples from all groups: between 17.5 - 32.5 % in the S1, S4 and restricted-fed birds from Chapter 6. The levels were even higher in the samples from the lame birds (52.6 %), and the *ad libitum*-fed birds from Chapter 6 (56-81%). This was reflected in the particularly high rbc counts from the smears made from the latter birds. Based on these results, it might be expected that the synovial tissue of the latter birds was more inflammed, however this was not reflected in the CI and SL scores, which indicated only mild inflammatory changes.

Older blood e.g. from chronic haemorrhage, appears as dark yellow or pale amber colour due to breakdown of the red blood cells and accumulation of bilirubin compounds (Fernandez *et al*, 1983; Houlton, 1994). The presence of haemosiderin-laden macrophages and erythrophagia also confirms previous haemorrhage (Fernandez *et al*, 1983). Between 0-12% of the samples from the S1, S4 and restricted-fed birds from Chapter 6 had colouration suggestive of old blood. The level was slightly greater in the lame group (18.4%), but dramatically increased in the *ad libitum*-fed birds from experiment 7 (56-62%). The latter group of birds also had the highest median ghost cell counts, most of these cells being degenerating red blood cells.

Specific Gravity: The median specific gravity of the samples varied: those for the experimental birds in Chapter 6 were around the average value of 1.010 quoted for mammals (White *et al*, 1978). The samples from the S1 and S4 birds were higher, however, at 1.014 -1.015. Perman (1980), quotes a value of 1.010 (range 1.008-1.015) for bulls, but up to 1.015 (range 1.010-1.018) for swine (quoting Crimmins and Sikes, 1965). As expected, the mean specific gravity of the samples from the lame birds was much higher (1.026), which can occur due to the presence of cells, bacteria and fibrin.

pH: The median pH was surprisingly alkaline in some of the groups, ranging from 7.5 in the restricted-fed birds described in Chapter 6, up to 8.4 in the *ad libitum*-fed birds from the same experiment. This is considerably higher than the mammalian average of 7.3-7.4 quoted by White *et al* (1978). A range of studies quoted by Perman (1980) also report much lower values in other species: cattle = 7.31 (Bauer *et al* 1940) or 7.4 (Amrousi *et al* 1966); *post-mortem* human = 7.4 (Bauer *et al* 1940); dog = 7-7.8 (Sawyer 1963); swine = 7 - 7.2 (Crimmins and Sikes 1965).

The significantly higher pH of the synovial fluid in the *ad libitum*-fed birds is an interesting finding, as few other body fluids are so alkaline. The pH of avian blood is normally between 7.45-7.63 (Freeman, 1984). Most body fluids in mammals have maximum pH of about 7.5, only jejunal fluid (up to pH 7.8) and bile (up to pH 8), being more alkaline (White *et al*, 1978). The increased alkalinity of the synovial fluid might explain why birds get urate arthritis, as uric acid is more likely to form sodium urate crystals in an alkaline environment. Although urate arthritis can be induced by excesses of dietary protein (e.g. through *ad libitum* feeding), the condition is sporadic, and is thought to be due to susceptible birds having a defect in tubular secretion of uric acid (Austin and Scott, 1991; Riddell, 1991).

In contrast to the results of the present experiment, a correlation between low pH and increased WBC counts has been demonstrated in synovial fluid in mammals (Ward and Steigbigel, 1978). Diagnostically elevated lactic acid levels have also been reported in synovial fluid in most types of septic arthritis (Brook *et al*, 1978, quoted by Perman, 1980). It would therefore be interesting to investigate the effect of bacterial infections on the pH of avian synovial fluid in more detail. The more alkaline nature of the fluid would also have an important effect on the pharamacodynamics of drugs acting within the joints.

Several of the other tests proved less useful, and so the results have not been included. The mucin clot test required a relatively large amount of the fluid, and was very subjective, and therefore of questionable value. The results of the synovial fluid glucose assays were also very variable, probably because small amounts of the fluid were divided between the tubes, and did not mix well with the preservatives. As the glucose levels did not appear to differ significantly between the plain, heparin, and oxalate samples, however, the entire sample could in future be put into heparin (as heparinised samples can also be used for protein assays). The tests also required very small quantities of sample to be pipetted: while this is not a problem for serum, it proved difficult with the viscous synovial fluid. In general however, the glucose levels were much higher in the serum than the synovial fluid. This might be due to either the excessive food intake of the broilers, or perhaps increased cellularity of the joints due to low grade chronic inflammation or infection (Perman, 1980).

As would be expected, the synovial fluid of poultry appears to be very similar to that of mammals. An interesting difference is the marked alkalinity of synovial fluid in *ad libitum*-fed broilers in comparison to most other body fluids. From a welfare aspect however, the most important finding is the high percentage of samples from the *ad libitum*-fed broilers that contained blood. This study has demonstrated that visual assessment and a few simple tests can help to distinguish between various pathological conditions in the joints in poultry.

CHAPTER 8:

FORCE PLATE ANALYSIS OF THE GAIT OF LAME BROILERS

8. 1 INTRODUCTION

The penultimate chapter of this thesis presents the results of a pilot trial in which a small number of lame broilers were subject to gait analysis using the forceplate. The ground reaction force patterns of the lame birds showed several interesting differences to those of sound birds. Analysis of the GRF traces with reference to the simultaneously recorded videotapes enabled the events that produced the forces to be identified and described. Due to the small sample size, statistical analysis was not performed on the data; instead, the results are presented as case studies. The results are therefore descriptive, rather than statistically significant, but serve to demonstrate several ways in which birds alter their gait and GRF's in lameness.

Five lame broilers were selected from a local commercial poultry flock; the birds had been raised under standard commercial conditions, and the flock was due to be culled the following day. The subjects were chosen by walking through the broiler shed and selecting birds that moved out of the way more slowly than the others. Four birds that showed moderate difficulty walking, and one with a more severe unilateral lameness, were selected for testing. The birds, which were not thought to have been lame for more than a 'few' days, were then moved to the testing premises at SAC, Aberdeen, under special licence from MAFF.

The trial was carried out using the equipment and testing set-up described in Chapter 8, and illustrated in Figure 2.6.

The birds were allowed to rest overnight on deep litter, with *ad libitum* access to feed (Roslin Broiler Starter diet) and water. The following morning, they were placed in the testing run, and gently encouraged to walk along it back towards their home pen, to allow them to become familiar with the environment. Between 5 and 10 runs per bird were collected in the afternoon, the number of runs depending on the willingness of the bird to walk (which reflected the severity of its lameness). As well as the force plate and video recordings, a simple visual assessment was made of each bird's gait. The degree of lameness was scored on a scale of 0 to 5, where 0 = sound and 5/5 was severely lame or refusing to move. The ground reaction force data from three runs from each bird are presented, along with a 'typical' GRF trace. All three traces are presented for bird 1, annotated to indicate the events producing the forces, which are then described in more detail in the text.

After testing was finished, the hocks were examined, and then the birds were killed by an overdose of anaesthetic (Pentobarbitone sodium, Merial Animal Health). The birds were then weighed and subject to *post-mortem* examination. The protocols for collecting and assessing the various samples have been described previously:

- synovial fluid (Chapter 7.2 and Appendix 7.1)
- synovial membrane (Chapter 7.2 and Appendix 4.3)
- tibiotarsi (for measurement of torsion and tibial plateau angle, and subsequent histological examination for TD lesions) (Chapter 4.2 and Appendix 4.3).

In the present experiment, to investigate possible cause(s) of the various lamenesses, synovial membrane samples were also taken from the hip and stifle joints, and sections of the proximal and distal epiphyses of the femur, tibiotarsus and tarsometatarsus were examined for lesions.

8. 2 CASE STUDIES

BIRD 1 (Bodyweight 2.77kg).

This bird was the most severely lame (5/5), and would not walk without encouragement, being very reluctant to take weight on its right leg. Both hocks had marked heat in them, and the right hock was slightly swollen. Neither hock appeared bruised, and both had normal ranges of movement (without crepitus).

Post-mortem examination:

A larger amount of fluid was obtained from the right hock (0.7ml) than the left hock (0.5ml), but the gross appearance of both samples was similar – both were cloudy with old blood. The viscosity was normal, and the pH 8.5. Differences were apparent between the two samples on cytological examination, however. While the sample from the left hock contained only a few red blood cells (5/5 cells), that from the right hock was much more cellular. Of the 295 cells counted, 255 were monocytes or macrophages, which is typical of an early inflammatory response (mononuclear cells predominating after about 24 hours).

Examination of the synovial membrane samples from the joints of the left leg showed only mild changes in the synovial layer, with a mild cell infiltrate. This was also the case for the sample of synovial membrane from the right hip. The samples from the right stifle and hock, however, both showed marked (score 3) synovial layer thickening, and moderate (score 2) subsynovial infiltration.

Interestingly, this bird had lesions in the proximal epiphysis of both femurs, apparent on histological examination. The lesions appeared as amorphous, avascular areas of basophilic cartilage, arising from the epiphyseal cartilage in the region of the femoral

head. These are similar to the dyschondroplastic lesions described by Duff (1984 a,c). The proximal femoral cartilagenous epiphysis was still attached, and there were few physeal clefts, suggesting this was not traumatic (Duff and Randall, 1987). The extensive vascular occlusion described for femoral epiphyseal infarction was absent (Duff, 1984b).
Both the degree of tibiotarsal torsion and the tibial plateau angles were within the normal ranges.

Video analysis:

The bird tended to either stop and stand holding the right leg up, or try to fly off the plate. When persuaded to walk, the right leg had very short stance periods, and very long swing periods, when it was held up in the air. In contrast, the sound limb had very long stance periods, and very short swing periods, so that it was replaced on the ground as soon as possible. As the bird preferred to keep the right leg off the ground, the periods of double contact were also very short. An interesting behaviour was demonstrated by this bird, which appeared to flap its wings prior to and during placement of the lame leg on the ground. This had the effect of raising the body upwards, which would obviously reduce the vertical load on the lame leg.

Ground reaction force measurements:

Figure 8.1: GRF trace of run 19. Period marked '1' represents the swing phase of the sound leg and stance phase of lame leg. Period marked '2' represents the swing phase of the lame leg, and stance phase of the sound leg.

Figure 8.2: GRF trace of run 20.

Figure 8.3: GRF trace of run 40

Table 8.1a: Analysis of ground reaction forces over runs from bird 1

run	19	20	40
speed (m/sec)	0.14	0.08	0.08
cadence (steps/min)	103.45	60.50	61.02
vertical loading rate (bw/s)	3.41	0.60	14.41
peak vertical force (%bw)	181.36	167.41	168.78
minimum vertical force (%bw)	26.03	55.34	30.68
peak propulsion force (%bw)	48.38	29.74	27.18
peak braking force (%bw)	48.38	28.33	27.65
Y ratio	1.00	1.05	0.98
'maximum' mediolateral force (%bw)	40.37	26.53	47.08
'minimum' mediolateral force (%bw)	15.21	20.98	19.14
X ratio	2.65	1.26	2.46

Table 8.1a shows that both the speed and cadence were predictably slow in this very lame bird. The vertical loading rate in runs 19 and 40 was quite high, as the sound leg was first placed on the plate. In run 20, the lame leg was placed first onto the plate, and the vertical loading rate was much lower. The peak vertical forces are consistently higher than the

median values established in Chapter 6 for 6 week old *ad libitum*-fed broilers (124.6-126.7 %bw). The vertical force on the lame leg dropped as low as 26% of bodyweight. The peak braking and propulsive forces were equal over a run, resulting in a Y ratio of approximately 1. The mediolateral forces are very different over a run, however, resulting in X ratios ranging from 1.26 – 2.65.

Table 8.1b: Analysis of ground reaction forces over steps from bird 1

run	19	19	20	20	40	40	40
step	a	b	b	c	b	c	d
leg	lame	sound	sound	lame	sound	lame	sound
Fz max (%bw)	84.3	156.9	167.6	75	162.5	50.3	169
Fz min (%bw)	26.1	38.2	55.4	55.4	35.4	30.7	75.4
Fy max (%bw)	12.2	21.7	29.8	14.1	21.7	8.3	20.5
Fy min (%bw)	5.8	37.6	28.4	5.5	27	4.4	10.6
Y ratio	2.1	0.58	1.05	2.56	0.8	1.88	1.9
Fx max (%bw)	8.1	40.4	26.6	12.9	28.4	10.8	47.1
Fx min (%bw)	1.6	15.2	16.4	5.3	19.2	5.1	4.4
X ratio	5.1	2.7	1.6	2.4	1.5	2.1	10.7
stance time (sec)	0.1	0.84	3.12	0.11	2.63	0.1	3.08

Table 8.1b. The peak vertical force was greatest during the stance period of the sound leg, decreasing to approximately half during the stance period of the lame leg. The vertical force drops quite low (Fz min) on both the stance leg and the sound leg. The craniocaudal braking and propulsive forces were variable in the sound leg, however the braking force was almost twice the propulsive force in the lame leg. Both the peak mediolateral propulsive and mediolateral braking forces were lower in the lame limb, compared to the sound one. There was also a very obvious difference in stance time – the lame leg being in ground contact for a much shorter period than the sound one.

Discussion:

Although this bird had lesions in both hips, dyschondroplasia does not always cause lameness (McNamee *et al*, 1998). It is equally possible that the lesions could cause a mild bilateral lameness, which would then have been difficult to detect. The dramatic lameness in the right leg, however, is most likely to have arisen from the inflammatory process occurring within the hock joint.

The 'abnormal' aspects of the gait pattern are illustrated most dramatically in the third trace (run 40). The sound leg is loaded rapidly at the start of the trace (Fz rate = 14.41 bw/s), after which the bird spends a prolonged period one-legged standing. When encouraged to take a step, the right leg is placed down, but by flapping its wings to raise its body off ground, the bird reduces the vertical load to well below bodyweight (30.68% bw). Examination of the simultaneous videotape record shows that the peak before this trough

appears to arise from the action of the left leg pushing the body upwards, prior to the lame leg being placed down. The second peak, immediately following the trough, occurs as the sound leg is replaced on the ground as quickly as possible, and the bodyweight rapidly loaded onto it. This results in peak forces greater than the usual range seen in normal walking – in this instance, up to 169.8% bodyweight. Thus the bird hesitates to put the lame leg down, pushes up on the sound leg to raise the body, and flaps wings to keep it up while the lame leg is in ground contact, then 'lands' heavily on the sound leg again in the next step. The bird then has another period of standing with the lame leg raised. This pattern is then repeated.

The craniocaudal force traces appear to differ between the lame and sound leg. Although it is quite easy to identify the craniocaudal force traces for the sound leg, they are obviously different to those of a normal bird walking at a steady speed across the plate. Again referring to Figure 8.3, a sharp negative braking force occurs as the sound leg is put down (step B), and then there is a long pause as the bird stands on that leg. The propulsion force then occurs, as the body is advanced before the other leg is put down. The craniocaudal force trace of the next step (step C), on the lame leg, is more difficult to interpret, other than to note that it is very short and the magnitude is also very small. This reflects the short contact time of the foot with the ground, and suggests that the leg may be being used more as a supportive strut, than for any sort of body advancement. More detailed analysis of this part of the GRF trace would be possible if it could be replotted over an expanded timescale; unfortunately, this sort of manipulation is not possible in Bioware, but may be possible if the data was exported into another software package such as Excel. It is also interesting to note that although the speed was obviously not constant across the plate, the peak braking and propulsive forces over the runs are equal, with a Y ratio of approximately 1. In two of the three traces, the X ratio is much greater than one, however, which suggests that the bird did not take a particularly straight path across the plate.

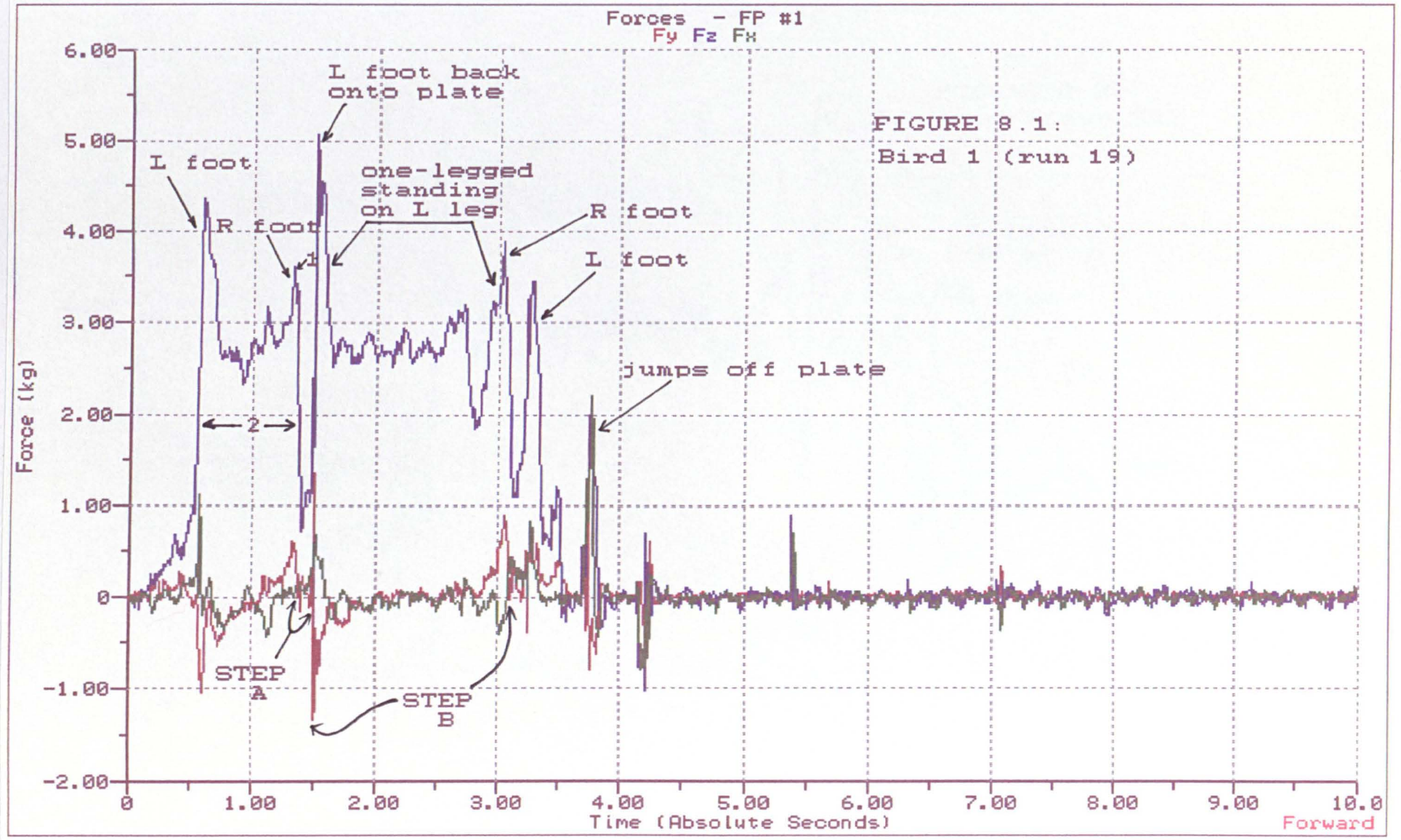


Figure 8.1: Ground reaction force trace from Bird 1 (run 19).

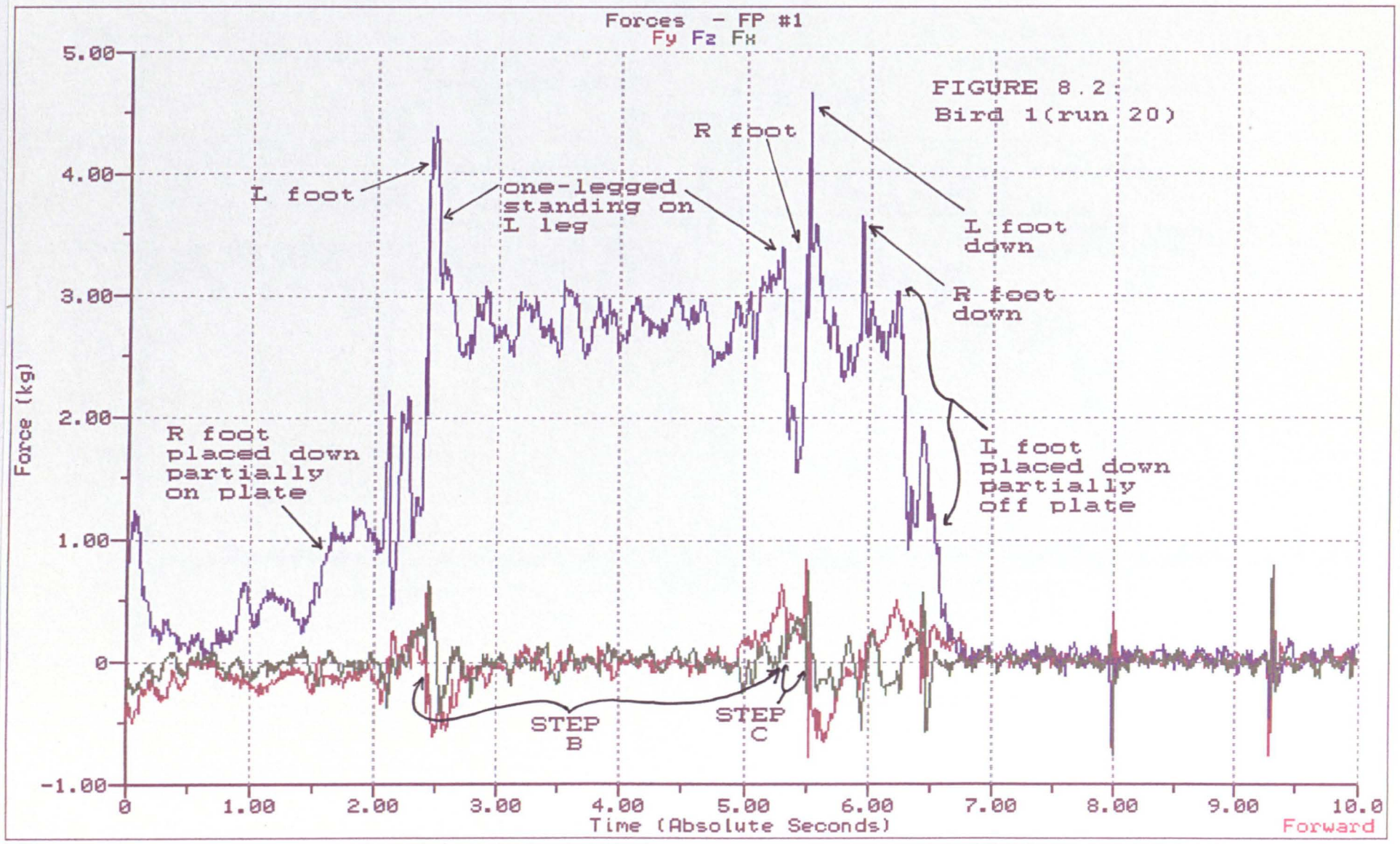


Figure 8.2: Ground reaction force trace from Bird 1 (run 20).

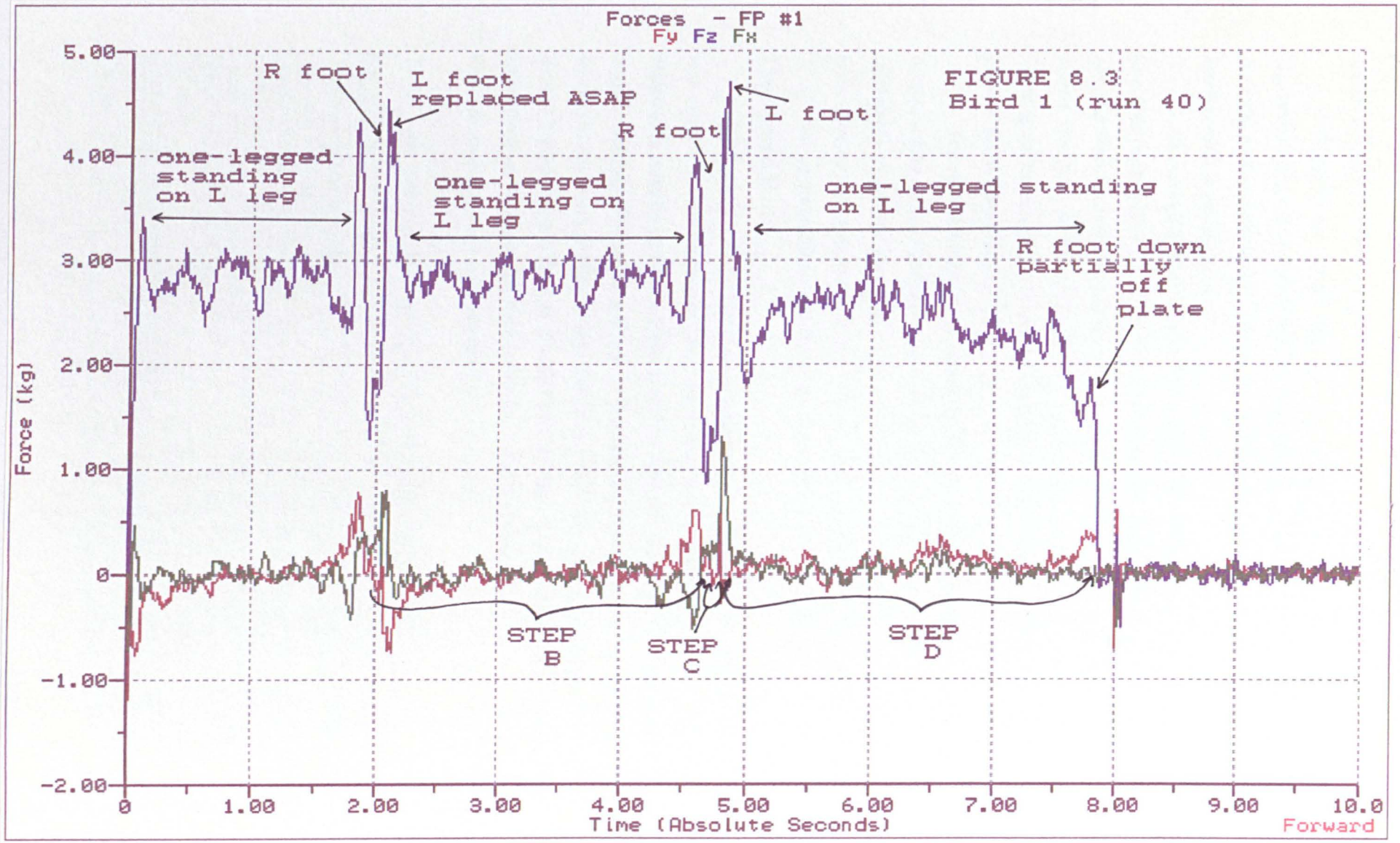


Figure 8.3: Ground reaction force trace from Bird 1 (run 40)

BIRD 2 (bodyweight 2.01kg).

This bird was quite willing to walk, and showed a less severe lameness on the right leg, graded as 3/5ths lame. Both hocks felt slightly warm, but neither was bruised or swollen, and both had normal ranges of movement (without crepitus).

Post-mortem examination:

The synovial fluid from the right joint (0.3ml) was slightly cloudy, medium yellow in colour, and normal viscosity. There was evidence of old blood, but no fresh blood, and pH of 8. The synovial fluid (0.6ml) from the left joint was also slightly cloudy and medium yellow in colour, with normal viscosity and pH of 8. There was no blood in this joint. Cytological examination of the synovial fluid found very few cells in either sample (3-4 ghost cells and red blood cells).

The synovial membrane samples from most of the joints showed only mild inflammation and cell infiltrate. The sample from the left stifle showed severe synovial layer thickening (with mild sub-synovial cell infiltrate) however, and the sample from the right hock showed moderate synovial layer thickening, again with mild sub-synovial infiltrate.

No abnormalities were found on examination of the bones.

Video analysis:

The severity of the lameness appeared to vary between the runs; sometimes the bird was obviously lame on the right leg, other times it seemed only stiff. The bird walked reasonably steadily across the plate however, without showing prolonged periods of one-legged standing. The stance periods for the lame leg were still very short, and accompanied by very short swing periods of the sound leg, which was quickly replaced on the ground next to the lame leg. Although this bird did not show wing flapping, it still appeared to raise its body up on its sound leg, before stepping onto the lame leg.

Ground reaction force measurements:

Table 8.2a: Analysis of ground reaction forces over runs from bird 2

run	10	12	32
speed (m/sec)	0.18	0.15	0.15
cadence (steps/minute)	94.49	140.00	120.00
vertical loading rate (bw/s)	2.74	1.64	4.01
peak vertical force (%bw)	139.60	144.74	138.30
minimum vertical force (%bw)	71.42	59.85	60.50
peak propulsion force (%bw)	17.56	18.15	24.54
peak braking force (%bw)	19.10	18.15	18.15
Y ratio	0.92	1.00	1.35
'maximum' mediolateral force (%bw)	24.59	25.84	26.83
'minimum' mediolateral force (%bw)	28.43	26.83	24.24
X ratio	0.86	0.96	1.11

Table 8.2a: the speed and cadence of this bird are both greater than those of bird 1, reflecting the less severe lameness. Again, the lame leg is loaded more slowly (run 12) than the sound leg (runs 10 and 32). It can also be seen that the peak vertical forces are not as high as those of bird 1, being only slightly above those found for both J-lines and broilers in previous experiments. Likewise, the vertical force does not appear to drop as low as it did in the runs by bird 1. Both the braking and propulsive forces are approximately equal over a run, as are the mediolateral forces, resulting in both Y and X ratios approximating 1 (in contrast to those of bird 1).

Table 8.2b: Analysis of ground reaction forces over steps from bird 2

run	10	10	12	12	32	32
step	c	d	b	c	b	c
leg	lame	sound	lame	sound	lame	sound
Fz max (%bw)	132.8	132.8	140.6	140.5	118.1	132.2
Fz min (%bw)	84.7	82.1	78.9	79.5	89.8	84.7
Fy max (%bw)	11.7	15.6	13.7	9.2	7.3	10.5
Fy min (%bw)	9.2	13.7	3.2	14	8.9	16.2
Y ratio	1.27	1.14	4.3	0.66	0.82	0.65
Fx max (%bw)	24.5	15.6	25.8	26.8	19.7	24.2
Fx min (%bw)	4.5	9.2	4.1	5.1	12.7	12.1
X ratio	5.4	1.7	6.3	5.3	1.6	2
stance time (sec)	0.41	0.84	0.31	0.49	0.43	0.75

Table 8.2b The peak vertical forces were similar between the lame and sound limbs (except on one step), as were the minimum vertical forces. Thus the differences in vertical force between the lame and sound limbs are not as obvious as in bird 1. This reflects the variable nature of the lameness in bird 2, and the fact that the pairs of consecutive steps were chosen at random from the run. Both the craniocaudal and mediolateral forces were very variable over the runs, neither ratio consistently being around 1. The stance period was consistently shorter for the lame leg, however.

Discussion:

Similar 'abnormalities' were apparent in the GRF traces of this bird to those of bird 1, although they were less marked. A 'typical' GRF trace from bird 2 is illustrated in Figure 8.4 (run 12). It appears that the bird was quite willing to load the lame right leg (2.74bw/s) at the start of the run. The peaks in the vertical force mainly occurred with the left (sound) leg on the ground, and the troughs with the right leg on the ground. The peaks are not as high, and the troughs not as low, as those of bird 1 however. The craniocaudal forces are more easily identified than those of bird 1, being more like those of normal birds, without the extended period of zero force when the bird is standing still.

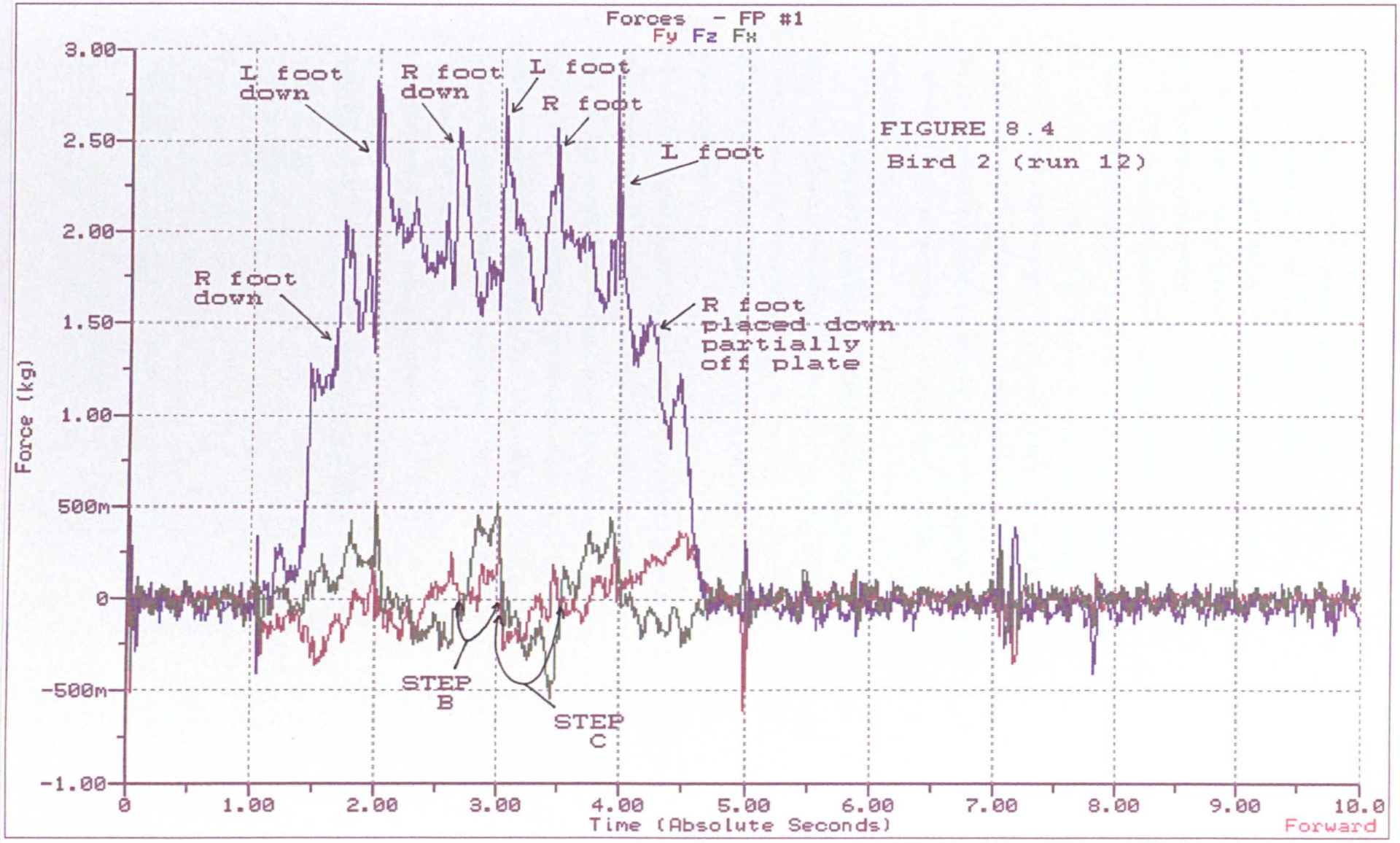


Figure 8.4: Ground reaction force trace from Bird 2 (run 12)

BIRD 3 (bodyweight 2.37kg).

This gait of this bird was similar to that of bird 2; it appeared to have a variable right leg lameness, but was quite willing to walk across the plate. There was slight heat in both hocks, and the right hock was slightly swollen, but neither was bruised, and both had a normal range of movement (without crepitus).

Post-mortem examination:

The synovial fluid from the left hock joint (0.4ml) was clear, medium yellow in colour, free of blood, pH 7.5 and normal viscosity. Few cells were found on cytological examination (20 cells, 13 of which were monocytes/macrophages, the remainder red blood cells). In contrast, the synovial fluid (0.3ml) from the right hock was slightly cloudy, with evidence of old blood. The viscosity was normal and the pH 7.5. Cytological examination of the fluid showed increased cellularity, typical of an inflammatory response: of the 105 cells counted, 81 were monocytes/macrophages, 5 were lymphocytes, and the remaining 19 were red blood cells.

Histological examination of the synovial membrane samples showed mild synovial layer thickening and cellular infiltration in all the joints, except for the left stifle, which had moderate synovial layer thickening, with a moderate sub-synovial cell infiltrate. No abnormalities were found on examination of the bones.

Video analysis:

The degree of right leg lameness was variable between runs, but appeared to be most pronounced (3/5ths lame) in run 1, when the bird seemed to 'drop' noticeably on the right leg. In contrast to the two previous birds, which reduced the load on the lame leg by variably flapping the wings, and pushing up on the sound leg, this bird demonstrated a third method –flexing the joints of the lame leg (without seeming to have previously thrust up on the sound leg). It showed no periods of one-legged standing, and made reasonably steady progress across the plate.

Ground reaction force measurements:

Table 8.3a: Analysis of ground reaction forces over runs from bird 3:

run	1	3	33
speed (m/sec)	0.09	0.08	0.09
cadence (steps/minute)	95.67	61.02	59.52
vertical loading rate (bw/s)	0.79	0.59	0.66
peak vertical force (%bw)	138.46	127.05	115.05
minimum vertical force (%bw)	59.97	82.88	86.69
peak propulsion force (%bw)	16.48	21.34	10.27
peak braking force (%bw)	15.38	19.74	13.78
Y ratio	1.07	1.08	0.75
'maximum' mediolateral force (%bw)	15.68	10.82	13.27
'minimum' mediolateral force (%bw)	15.68	15.98	10.57
X ratio	1.00	0.68	1.26

Table 8.3a: The speed and cadence of this bird were similar to those of bird 1, despite its apparently less severe lameness. The vertical loading rate was also very slow, that for the sound leg (0.79 bs/s) being only slightly greater than the lame leg (0.59-0.66 bw/s). The peak vertical forces were around the normal range, decreasing at times to 60% bw on the lame leg, and 87% bw on the sound leg. The craniocaudal forces were approximately equal in two of the three runs, however the mediolateral braking and propulsive forces were equal in only one run. This reflects the variability of the lameness between runs.

Table 8.3b: Analysis of ground reaction forces over steps from bird 3:

run	1	1	3	3	33	33
step	c	d	c	d	a	b
leg	sound	lame	sound	lame	sound	lame
Fz max (%bw)	119.7	126.8	126.8	97.4	114.8	110.5
Fz min (%bw)	89.8	95.8	86.5	85.4	86.5	89.8
Fy max (%bw)	7.5	5.9	21.3	7.3	10.3	7
Fy min (%bw)	7.3	3.2	6.4	3.2	8.1	7
Y ratio	1.02	1.8	3.3	2.3	1.27	1
Fx max (%bw)	15.6	13.7	15.9	10.8	7.8	13.2
Fx min (%bw)	4.8	2.4	8.3	3.4	2.9	3.2
X ratio	3.3	5.7	1.9	3.2	2.7	4.1
stance time (sec)	0.57	0.32	1.08	0.78	1.32	0.86

Table 8.3b: The peak vertical force is similar between the lame and sound limb, and in the normal range, except on step 3d, when it never reached fully bodyweight. The minimum vertical force remained above 85% of bodyweight, reflecting the less severe lameness of this bird. The propulsive forces appeared to be greater than the braking forces on both the sound and lame leg, suggesting that the bird was accelerating across the plate. The X ratios were greater than 'normal' on the lame leg, as a result of higher propulsive forces. As with all the other birds, the stance period was lowest for the lame leg.

Discussion:

The traces from this bird were more difficult to interpret. A 'typical' GRF trace from this bird is illustrated in Figure 8.5 (run 1). This was the run in which the bird appeared to be most lame, however the 'abnormal' patterns seen in the traces from birds 1 and 2 are not so obvious. On some steps, it appears that the lame leg actually produces a peak just after being placed down, as might be expected in a normal bird, however the vertical force then decreased, as would occur if the bird flexed the limb. The subsequent placement of the sound foot on the ground was accompanied by a typically high vertical force peak. Overall, however, the peaks and troughs were not as great as those of the previous birds. The stance times were predictably lower in the lame limb compared to the sound limb.

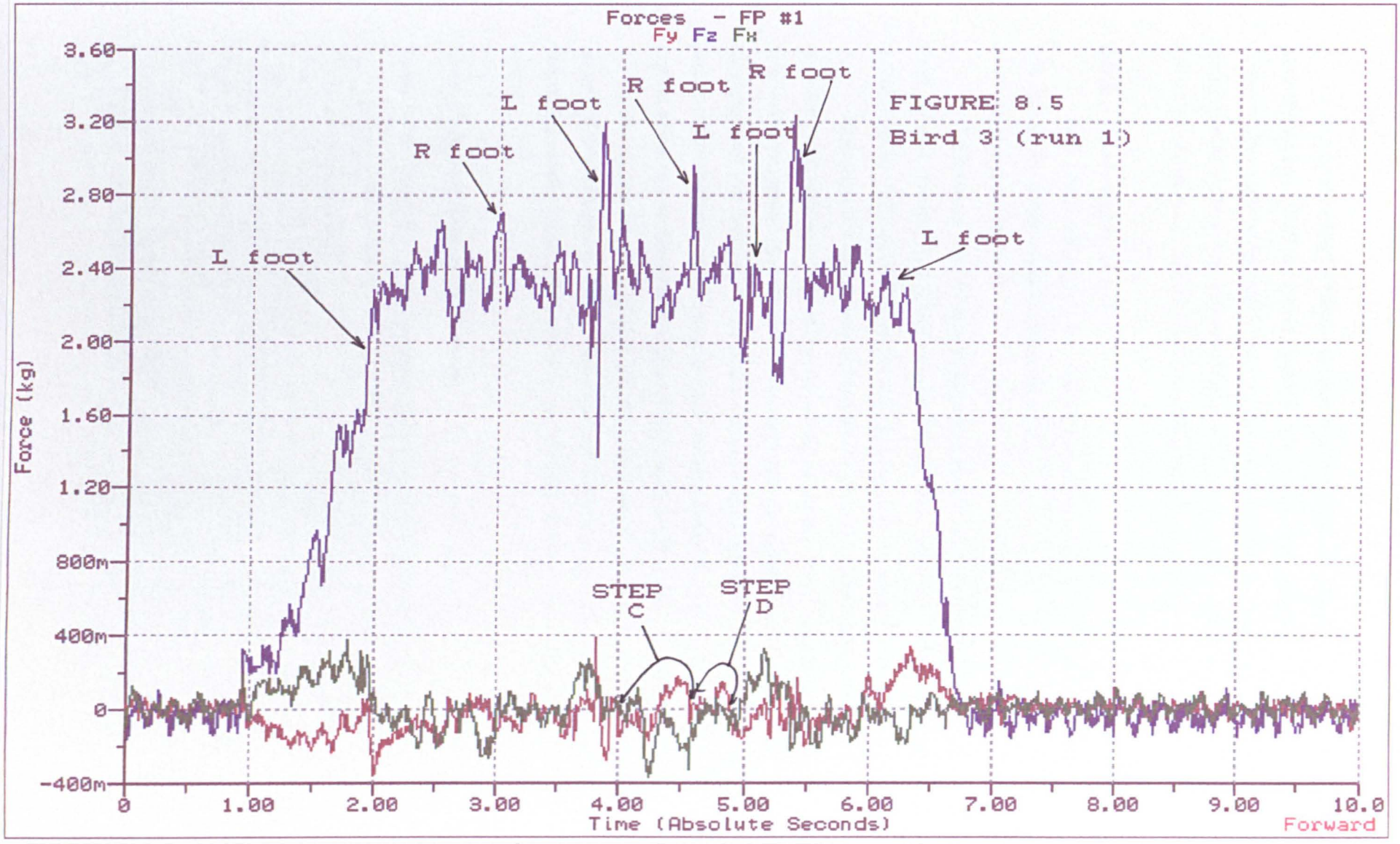


Figure 8.5: Ground reaction force trace from Bird 3 (run 1).

The two remaining birds appeared to be unsteady or ataxic, rather than obviously lame on one leg. Bird 5 was more unsteady than bird 4, and appeared to 'stomp' her feet down, while not obviously favouring one leg over the other. As might be expected, the GRF traces from these birds were more difficult to interpret, but they have been included to demonstrate potential limitations of force plate analysis on birds.

BIRD 4 (bodyweight 3.14 kg).

Both hocks felt slightly hot, but neither was swollen or bruised, and both had normal ranges of movement (without crepitus).

Post-mortem examination:

The synovial fluid from each joint was similar in gross appearance: 0.3mls of clear fluid, normal viscosity, pH 8, and no gross blood. There were few cells in either sample: 9 red blood cells in the sample from the left joint, and 2 in the sample from the right joint.

Histological examination of the samples showed mild synovial layer thickening in all joints, with normal to mild sub-synovial cell infiltrate. The epiphyseal cartilage of the left proximal tibiotarsus appeared wider than normal, but not typical of the type of lesion seen in dyschondroplasia. A large amorphous, avascular area of basophilic staining cartilage was present in the left proximal femur, however, arising from the epiphyseal cartilage. This is typical of the dyschondroplastic lesions described by Duff (1984 a,c), and was larger than the similar lesions seen in bird 1. In this case, however, the overlying articular cartilage was separated from the growth plate in the region of the lesion, although this could be a processing artefact. The tibiotarsal torsion and tibial plateau angles were within normal ranges.

Ground reaction force measurements:

Table 8.4a: Analysis of ground reaction forces over runs from bird 4

run.	5	6	7
speed (m/sec)	0.08	0.07	0.09
cadence (steps/minute)	65.34	63.16	72.00
vertical loading rate (bw/s)	0.59	0.92	2.30
maximum vertical force (%bw)	116.69	124.91	121.22
minimum vertical force (%bw)	82.19	80.54	80.12
peak propulsive force (%bw)	13.63	15.07	17.11
peak braking force (%bw)	14.46	10.99	10.39
Y ratio	0.94	1.37	1.65
'maximum' mediolateral force (%bw)	14.46	10.61	21.41
'minimum' mediolateral force (%bw)	15.10	24.88	11.82
X ratio	0.96	0.43	1.8

Table 8.4a: the unsteady gait of this bird was reflected in its slow speed and cadence. The vertical loading rate was lower on the right leg (0.59bw/s) compared to the left leg (0.92-2.3bw/s). The peak vertical forces were in the normal range, and decreased to around 80% of bodyweight. Run 5 had the most normal Y and X ratios. In the other two runs, the propulsive forces exceeded the braking forces, and the X ratios were also greater than 1.

Table 8.4b: Analysis of ground reaction forces over steps from bird 4

run	5		6		7	
step	a	b	c	d	a	b
leg	left	right	right	left	left	right
Fz max (%bw)	116.7	108	116.2	102.7	113	108.8
Fz min (%bw)	87.1	91.6	87.1	81.4	92.8	90
Fy max (%bw)	4.7	1.6	15.1	6.7	8.9	6.5
Fy min (%bw)	8.5	7.5	5.1	4.1	10.4	8.6
Y ratio	0.55	0.21	2.96	1.63	0.86	0.76
Fx max (%bw)	9.8	5.5	15.6	11.8	11.8	10.2
Fx min (%bw)	2.8	1.6	2.9	2.2	7.1	1.4
X ratio	3.5	3.4	5.4	5.4	1.6	7.3
stance time (sec)	0.95	0.7	0.86	1.14	0.93	0.68

Table 8.4b shows that the difference between peak vertical force and minimum vertical force is not as great as in the birds with an obvious unilateral lameness. The craniocaudal forces are variable, although they suggest that the bird was accelerating in run 6, and decelerating in runs 5 and 7. The X ratios are closer to one than many of the other birds, as might be expected (as the gait was bilaterally uneven). The stance times are variable between the legs, and there is no clear indication that one leg is being preferred over the other.

BIRD 5 (bodyweight 2.86 kg).

There was slight heat in both hocks, but no swelling or bruising, and both had normal ranges of movement (without crepitus).

Post-mortem examination:

The synovial fluid (0.3ml) from the left hock was clear, of normal viscosity, pH 8.5, with a trace of new blood, but no old blood. Of the 46 cells counted on cytological examination of the fluid, 25 were monocytes / macrophages, and 21 were red blood cells. The synovial fluid (0.3ml) from the right hock was cloudy with old blood, but was of normal viscosity and pH 8. As in the left hock, cytological examination of the fluid showed only monocytes / macrophages (26 out of 34 cells) and a few red blood cells (8 out of 34 cells).

Histological examination of the synovial membrane samples showed mild synovial layer thickening in the left hip, right stifle and right hock; the samples from all the other joints were normal. No abnormalities were found on examination of the bones.

Ground reaction force measurements:

Table 8.5a: Analysis of ground reaction forces over a run from bird 5

run.	15	24	25
speed (m/sec)	0.06	0.15	0.11
cadence (steps/minute)	68.70	116.13	118.52
vertical loading rate (bw/s)	0.54	3.20	1.82
peak vertical force (%bw)	128.03	129.40	128.94
minimum vertical force (%bw)	81.16	86.12	76.20
peak propulsive force (%bw)	18.11	23.91	18.98
peak braking force (%bw)	11.60	29.92	8.95
Y ratio	1.56	0.80	2.12
'maximum' mediolateral force (%bw)	16.99	17.90	20.34
'minimum' mediolateral force (%bw)	16.99	19.47	23.28
X ratio	1.00	0.92	0.87

Table 8.5a shows the higher average speeds and cadences for this bird. The vertical loading rates were higher on the left than the right leg, the peak vertical forces were in the normal range, and decreased to 76-80% bodyweight. While the Y ratios are very variable, the X ratios approximate 1.

Table 8.5b: Analysis of ground reaction forces over a step from bird 5

run	15		24		25	
step	a	b	b	c	a	b
leg	right	left	right	left	right	left
Fz max (%bw)	118.1	104.6	125.8	118.2	118.1	111.8
Fz min (%bw)	90.7	69.5	89.7	86.6	90.7	86.6
Fy max (%bw)	7.4	7.4	10.3	10.7	7.4	7.8
Fy min (%bw)	8.9	2.9	6.7	10.3	8.9	4.5
Y ratio	0.83	2.5	1.5	1.03	0.83	1.7
Fx max (%bw)	17	17	14.1	14.1	18.6	14.3
Fx min (%bw)	7.4	8.1	4.5	2.2	0.67	9.2
X ratio	2.3	2.1	3.1	6.4	27.7	1.6
stance time (sec)	0.72	0.62	0.62	0.87	0.59	1.31

Table 8.5b: the peak vertical forces were within the normal range, and decreased to 70%bw (still much higher than those of the very lame birds). Both the Y and X ratios were very variable – while the Y ratios were not close to the 'steady walking' value of 1, the X ratios (apart from 25a), were similar to those of normal broilers. The stance times were similar for each leg, except in run 25, where the stance period for the right leg was considerably shorter.

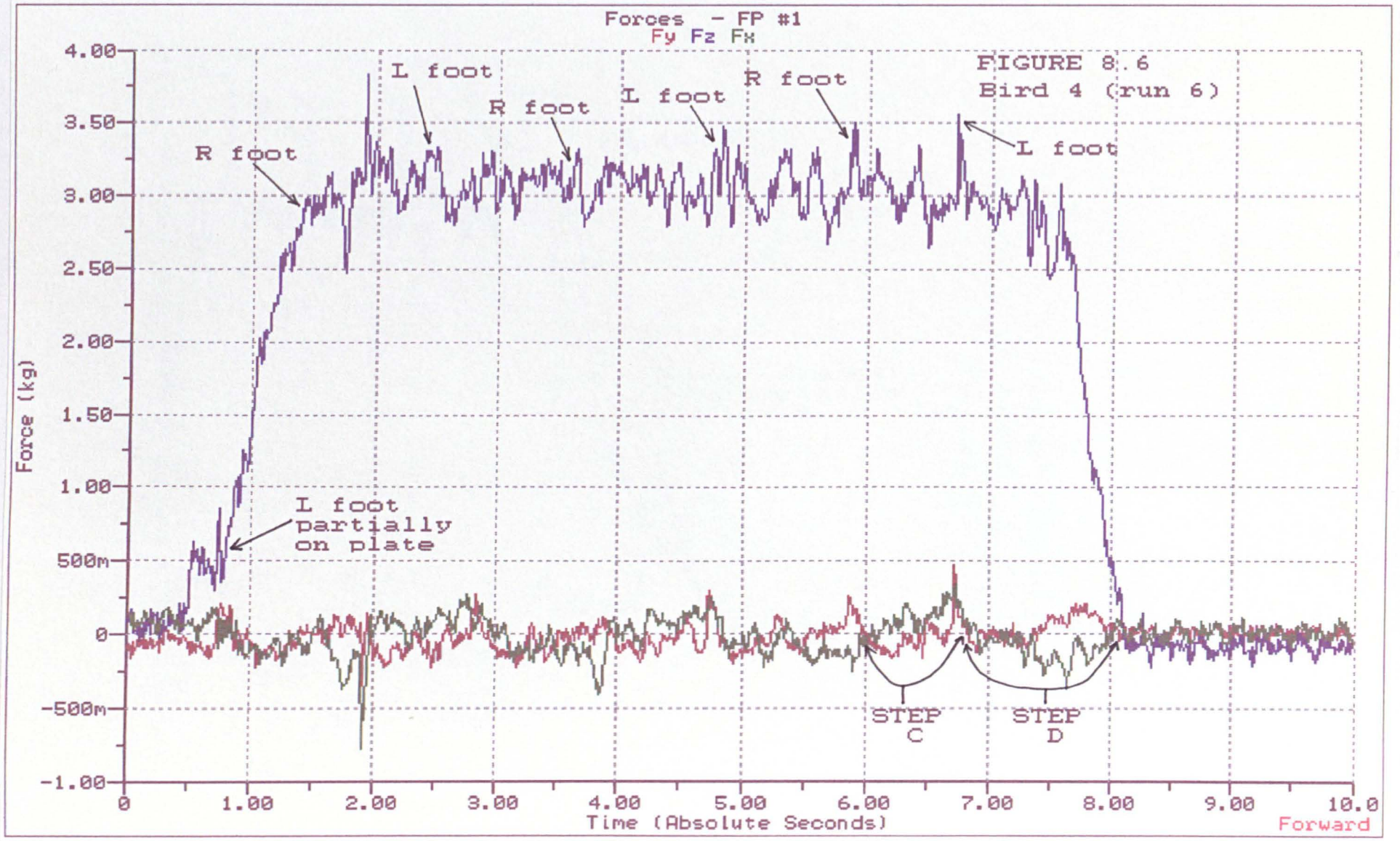


Figure 8.6: Ground reaction force trace from Bird 4 (run 6).

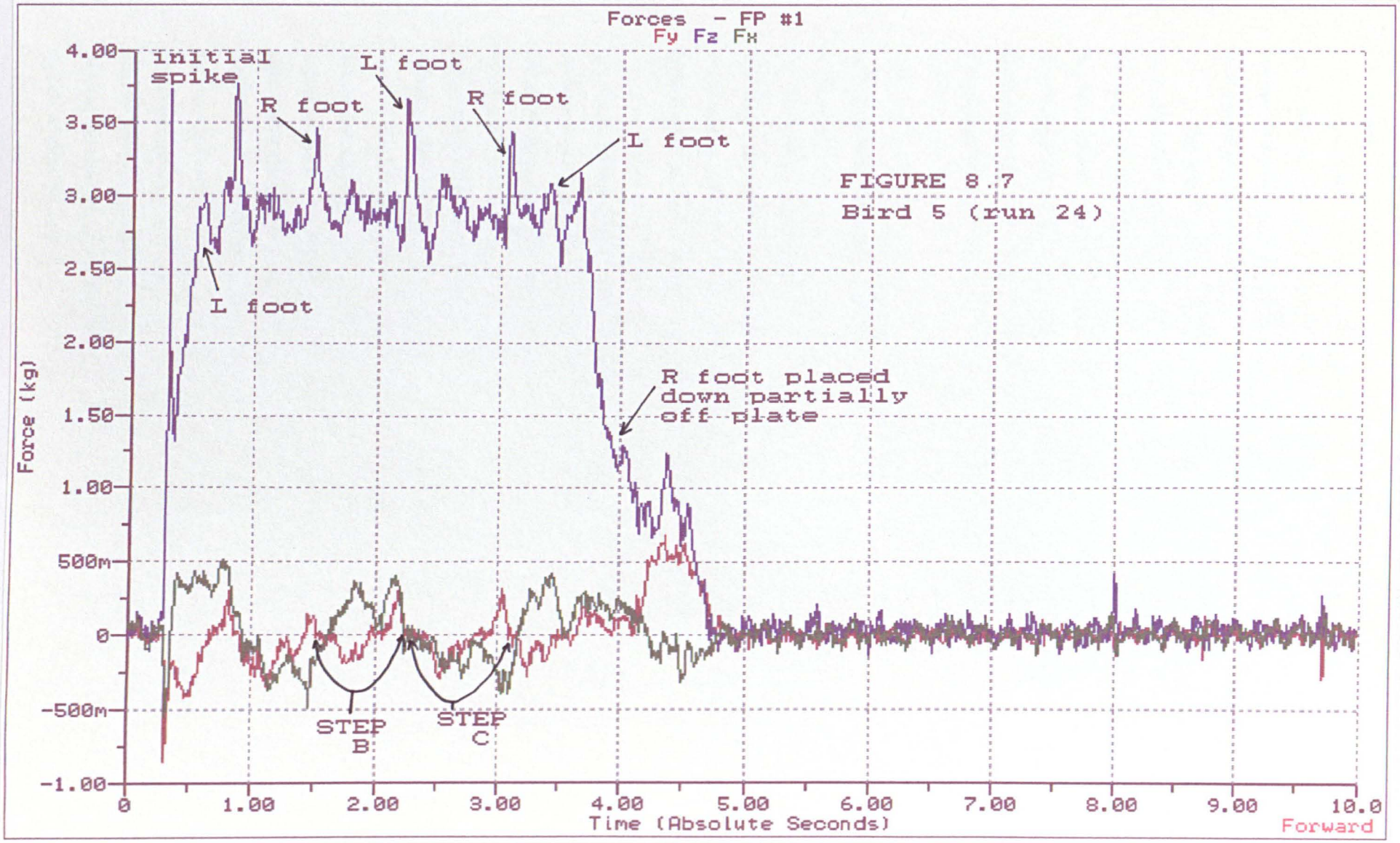


Figure 8.7: Ground reaction force trace from Bird 5 (run 24).

Discussion:

It is surprising that the large lesion in the left hip of bird 4 did not appear to cause an obvious left leg lameness. It is possible that the bilateral nature of the 'unsteady' gait pattern of this bird might be due to a concurrent problem in the right leg, which was undetected on examination.

The traces from these two birds do not appear to be very different from those of normal birds. Figure 8.6 (run 6) illustrates a 'typical' trace from bird 4, and Figure 8.7 (run 24) is a trace from bird 5. Examining trace 024 in more detail, the initial spike is an artefact, but subsequent vertical force peaks can be seen as each foot is placed on the ground and then loaded. In both the traces, the vertical peaks are not abnormally high however, and dramatic troughs are absent. It is also easier to recognise the more typical craniocaudal force patterns, and the mediolateral forces are predictably large.

8. 3 GENERAL DISCUSSION

The results obtained from the few birds in this experiment may not be representative of a larger population of lame birds, and it is not intended to present them as such. This study was intended to identify ways in which the ground reaction forces of broilers change in lameness, and to assess how difficult it would be to interpret the resulting traces.

In general, the speeds and cadences were lower in these lame birds than for most of the previous birds tested. The fact that speed decreases in lameness is well recognised (Andriacchi *et al*, 1977; Steven *et al*, 1983), however the effect on cadence is more variable. The finding that cadence decreased in the lame birds in this study may reflect the importance of cadence in speed of birds, as other subjects with disabilities may slow more by decreasing step length, e.g. people with Parkinsonism (Murray *et al*, 1969).

The stance times were considerably higher for the sound leg than the lame leg, with the reverse applying to the swing periods. This is a typical antalgic gait pattern, aimed at reducing the amount of pain experienced by the lame subject during walking. In the very lame birds, the gait pattern is obviously asymmetrical between the limbs, but quite regular between one step and the next, as described by Whittle (1991). In some instances, the antalgic gait was taken to the extreme, the birds holding the lame leg off the ground for prolonged periods (one-legged standing). Thus in the lame birds, the periods of double contact were often short, showing that these birds preferred periods of instability to periods of pain induced by weight-bearing. In humans with such a degree of lameness, canes or crutches might be used to assist walking and improve stability, while avoiding weight

bearing on the painful limb. It was also apparent from the videotapes that the birds tended to place the sound leg on the ground next to the lame leg, which would mean that the step length for the sound leg would be relatively short. Again this is a typical characteristic of a pathological gait (Whittle, 1991), and would reduce both the time spent in single support by the lame leg, and the propulsive forces it would need to generate.

While changes in the GRF patterns were obvious in the three birds with predominantly unilateral lamenesses, they are less obvious in those with bilateral problems e.g.'wobbly' or ataxic birds. In the lame birds, the most obvious changes in the GRF's were apparent in the vertical forces; both the peak forces, and the loading rates. The vertical loading rate tended to be lower in the lame leg than the sound leg, which was obvious from the reluctance of the bird to use the leg (the birds tended to delay loading the lame leg, often holding it up off the ground). Vertical loading rates can also be low on the sound leg if the bird is moving slowly (as demonstrated in previous studies). The most obvious differences showed up in the peak vertical forces: peak vertical forces on the lame leg were often below bodyweight, and sometimes dropped as low as 30% bw, as the birds attempted to minimise the load on the lame leg. The peak vertical forces on the sound leg appeared to show a 'compensatory' increase, so that they were often considerably higher than the normal range. Similar findings have been described in other species (Budsberg *et al*, 1988; Merkens and Schamhardt, 1988b; Jevens *et al*, 1996).

Changes were also apparent in the craniocaudal forces, often making it difficult to identify individual footsteps. The most striking abnormality occurred when the birds stood on one leg for prolonged periods, so that the initial braking force was separated by a long delay from the propulsive force. This would be a period of considerable instability, during which the bird would need to keep its CG positioned above the stance leg to maintain its equilibrium. In general, prolonged periods of single support tend to be avoided in subjects where stability is particularly important e.g. the very young and very old (Murray *et al*, 1969; Todd *et al*, 1989; Whittle, 1991). However certain types of normal birds often stand intermittently holding one leg up (at rest), and this may be easier in birds with relatively large feet (greater area within which to position the CG) (Alexander, 1982). While this behaviour was frequently observed in resting Brown Leghorns, it was not observed in any of the 'normal' broilers in the previous experiments.

Patterns varied for both the craniocaudal and mediolateral forces, however the trend was for:

- the braking and propulsive forces to be approximately equal over a run (8/12 runs), even although the speed was not steady. This could be explained if the forces produced during the prolonged stance periods of the sound limb are 'balanced' by those

produced during the brief stance periods on the painful limb e.g. if the bird decelerates strongly, the propulsion component can be absent (and vice versa) (Jayes and Alexander, 1978).

- the Y ratio to be greater in the lame than the sound leg over consecutive steps (7/9 steps). This means that the propulsive forces were greater than braking forces, possibly because birds were reluctant to place the lame leg down and tended to decelerate it a lot before doing so, and then quickly pushed the bodyweight back onto the sound leg again.
- the mediolateral forces to be very variable over a run,
- the X ratio to be greater in the lame than the sound leg over consecutive steps (7/9 steps). Thus the propulsive force was greater than the braking force, probably for the same reason that the Y ratio is greater in the lame birds.

The changes in the vertical force were particularly dramatic, and they were also very repeatable in the lame birds. Analysis of the simultaneous videotapes made of the runs enabled the ways in which the forces were produced to be described. The birds appeared to use three different techniques for reducing the vertical force on the lame leg, depending on the severity of the lameness:

a) the most severely lame bird (1) flapped its wings to raise its body mass up, immediately prior to, and during, the stance period of the lame leg. Raising the CG in this way is obviously a very effective way of reducing the vertical load on the lame leg, but is very wasteful of energy: as discussed previously, abrupt changes in momentum (e.g. the direction of motion) result in high energy costs (Saunders *et al*, 1953). In such a case, the bird has to produce a force greater than its mass x gravity to raise its body. Thus the bird creates its own floating phase (otherwise only seen in hopping and running). It has also been demonstrated that birds can use their wings very effectively to decelerate their body just prior to landing, and so decrease the impact forces (Bonser and Rayner, 1996).

b) the birds pushed upwards on the sound leg, to raise the CG prior to stepping onto the lame leg. As discussed previously, the body effectively falls forward under gravity, and so if the CG starts higher, there will be slightly more time before the stance leg begins to decelerate and support it (the dynamic equilibrium). During this time the sound leg can be rapidly swung forward and replaced into ground contact. Again, exaggerating the vertical excursion of the CG in this way is very energy inefficient.

c) the birds flexed joints of the lame leg as the bodyweight was loaded onto it. This is similar to the action of the knee in human walking, which bends slightly during mid-stance, temporarily unloading the limb, producing the minima between the two

maxima seen on the human vertical force trace (Whittle, 1991). This appears to be an example of birds using a human optimisation to more extreme effect. Knee flexion during stance in human walking serves to keep the CG low as it passes in an arc over the hip (Whittle, 1991), however in birds, it serves to lower it further to 'unload' the leg. (A similar reduction in vertical force has been demonstrated as birds bend their knees prior to take off (Bonser and Rayner, 1996)). It is well recognised that the most effective method of protecting the body from harmful impulsive loads is to flex the joints (particularly the knee and hips) e.g. when landing from a jump (Collins and Whittle, 1989). The birds appear to be applying this principle to the lame leg. As the CG is then lower than normal, a greater vertical force has to be applied to raise it again, which contributes to the peak in the vertical force seen when the subsequent sound limb is replaced on the ground. Most of this large vertical force probably results from the rapid rate at which the sound limb is replaced on the ground however. This could be a slightly more efficient adaption in the birds, if it involves some storage and recovery of energy in the elastic elements of the limb.

Previous work in this thesis seemed to indicate that birds did not use gait optimisations similar to those of humans during normal walking. The observations made in the present study suggest they may use some of them in pathological situations, however, and add some of their own such as wing flapping. As with humans, joint flexion seems to be particularly important: in the human, the knee is the most important joint for this purpose, and loss of knee function is considered to be the most costly pathology (Saunders *et al*, 1953). It seems likely that the same applies to birds (as hip movement is fairly limited), however the ankle joint may be equally important, due to the elongation of the tibiotarsus and metatarsus giving the joint a larger range of movement in birds. As discussed in the Introduction, abnormal movements can be useful if they enable the subject to compensate for an underlying problem. The exaggerated movements consumes excess energy, however, resulting in fatigue (Whittle, 1991), which explains the lower speeds.

This study also highlighted several difficulties in force plate analysis of gait in lame birds. The first was the difficulty in getting lame birds to walk. Although these birds were not as familiar with the environment as birds in previous experiments, they were even less motivated to walk because of their disabilities. While increased familiarisation with the testing set-up might help, dramatically altering the stimulus to walk e.g. by using food rewards or aversive conditioning, would alter their motivation, but is also likely to change their gait patterns (Wetzel and Stuart, 1977; Gentle and Corr, 1995).

The second problem was the difficulty in identifying individual steps within a run, both to make measurements over a single stance period, and to identify which step was produced by which leg. Although evidence exists for limb dominance in birds (Duff and Thorp, 1985a, Duff, 1986), it was unnecessary to distinguish between right and left steps in the previous work on sound birds, as the steps were selected randomly, and so there should be even numbers of each in the data sets. Determining which leg produced a particular step would obviously be important in lame birds, however. Although taking two consecutive steps per run would ensure equal numbers of right and left steps were included in the data set, identifying them (and distinguishing between them) requires examination of simultaneously recorded, accurately time-stamped videotapes.

In general however, the study demonstrated some interesting ways in which birds adapt their gait in lameness, which merit further investigation in a properly designed experiment.

CHAPTER 9:

GENERAL CONCLUSION

The aim of this thesis was to develop an objective method of gait analysis for poultry, and to describe and quantify various gait parameters. It was hoped to quantify differences between the gait of 'normal' chickens (Brown Leghorns) and that of broilers, and to determine whether these differences could be explained by the conformation of the birds, or the presence of pain.

Both of the systems used in this thesis proved to be effective methods of measuring gait in birds, each providing different types of information. The pedobarograph provided information on spatial and pressure measurements, while the Kistler force plate (KFP) enabled the magnitude and duration of the three-dimensional ground reaction forces to be quantified.

All the gait parameters measured were very variable, both between birds, and to a slightly lesser extent, within birds (with equal or greater variability between steps within a run as between runs). Despite this, many significant differences were identified between the gait patterns of Brown Leghorns and broilers (of different strains, and different growth rates).

BROWN LEGHORNS:

As speed increased, both cadence and step length increased. Over the range of speeds seen in this study, the birds appeared to increase speed more by increasing their step frequency, than the length of their steps. Step width and step angle were not significantly affected by speed, and it was observed that normal birds walked with the toes pointed inwards.

Plantar pressures were measured and pressure distributions plotted, and it was apparent that the pressure was concentrated in the areas of the digital pads. The highest pressures were found on the back toe (146-195 kNm^{-2}), and medial toe (149-218 kNm^{-2}), and the lowest pressures occurred on the metatarsal pad (16-131 kNm^{-2}). From these pressures, maximum (net) vertical forces were estimated (116-145% bw), and found to be of the same order of magnitude as those produced in human walking.

Ground reaction forces were measured using the KFP, and as with the spatial and pressure measurements, the forces were found to be very variable. The high variability of the measurements is probably due to fluctuations in speed as the bird takes several steps across the plate. Speed was found to have a significant effect ($p<0.01$) on many of the GRF parameters: peak vertical force, vertical loading rate, peak propulsive force, peak mediolateral force, braking and propulsion rates all increased with increasing speed. The effect of speed on peak braking force and Y ratio was more variable. In contrast, median stance time decreased with increasing speed ($p<0.01$). Speed did not have a significant

effect on X ratio, or on the braking or propulsion %'s, or integrals. The significant effect of speed on so many of the GRF's indicates that the results of such studies on birds must always be interpreted within the appropriate speed range.

The median peak vertical force range (125-150 % bw) agreed with the estimates obtained from the pressures measured on the pedobarograph. Peak propulsive forces (26.3-41.6 % bw) were greater than peak braking forces (20.3-25.6 % bw), and increased to a greater extent with increasing speed. A much greater push-off force is applied at higher speeds and the braking force, mainly due to friction on the floor, does not increase to the same extent. The rate of application of the force also changes: the propulsive rate was 4 times the braking rate at slow and medium speeds, but 6 times greater than the braking rate at fast speeds. As reported in other species, there was no change in the percentage of the cycle time spent in braking or propulsion, or in the braking or propulsion integrals, with change in speed. This reflects the fact that although the peak forces and rate of application of the forces increased with increasing speed, the stance times (over which the forces are applied) decreased. In contrast to most mammals, however, the birds produced consistently large peak mediolateral forces (10.4-22.1 % bw) during walking, and these increased significantly with increasing speed. This is a very inefficient way of walking, suggesting that the birds have ineffective gait optimisations for controlling lateral excursions of their CG.

BROILERS :

The gait patterns of two strains of broiler (relaxed and selected) were examined, and morphometric comparisons made both between, and within, the strains (the birds having been raised on different feeding regimes).

Growing most rapidly, the *ad libitum*-fed selected birds had the heaviest weights, greatest girths, longest legs, and widest tarsometatarsi of all the groups up to 6 weeks. At the same cull weight, however, the girths and tarsometatarsal widths were not significantly different between any of the groups. The *ad libitum*-fed selected birds had significantly shorter legs than the birds in the other groups, however, and therefore the greatest ratio of bone diameter / length (which increases strength).

Reflecting the selection criteria of the last 27 years, the selected birds had significantly greater breast muscle, thigh muscle, and breast/leg muscle ratios than the relaxed birds at cull. They also had significantly greater leg bone mass than the relaxed birds, but although the bones were significantly wider during growth, there was no difference in the width at cull. Within the strains, the restricted birds had greater leg bone mass than *ad libitum*-fed birds, reflecting the fact that slow growth allows for more bone development. The effects of selective breeding were also apparent, however, as the *ad*

libitum-fed selected birds (at 6 weeks) had greater bone mass than the restricted relaxed birds (at 24 weeks) at the same cull weight. The % ash content, an indicator of bone strength, increased with age, therefore the *ad libitum*-fed selected birds were carrying the greatest bodyweight on the weakest bones. Thus, although the selected birds had wider tarsometatarsal diameters than the relaxed birds (an increased surface area for loading should decrease the stress on the bones), the *ad libitum*-fed selected birds had the lowest % ash, suggesting the poorest quality bone. This suggests that the bone geometry, rather than mineralisation, has adapted to the increased stresses in these birds.

Gait analysis showed that all the broilers moved in the slow speed range of the Brown Leghorn birds. There was no significant change in speed with age: as the mean cadence decreased with age in all the groups, the mean step length increased (as leg length increased). Comparisons within strains between the different feed groups indicated that speed of movement was related to bodyweight, rather than motivation to find food. Most significantly, the *ad libitum*-fed selected birds moved with the slowest speed of all the birds, and took the shortest steps, despite having the longest legs. Step width increased in all groups as the birds grew, however the selected birds had significantly greater step widths at cull weight than the relaxed birds (there were no differences within the strains). The *ad libitum*-fed selected birds had significantly greater step angles than any of the other groups at all times: their feet pointed outwards, in contrast to the other three groups, which turned their feet inwards. Comparing the gait cycles, the *ad libitum*-fed selected birds had significantly greater % stance times, and significantly lower % swing times than the other groups at cull weight. The double contact times were also significantly greater in the *ad libitum*-fed selected birds than either of the relaxed groups (but not from the restricted-fed selected birds).

The results of the morphometric and gait studies were then considered together to determine whether the gait patterns could be explained by the conformation of the birds. While the relaxed birds breast muscle mass was around the 'bird average' of 15%, that of selecteds was much greater, at 19%, which must displace the centre of gravity anteriorly. It is therefore possible that the *ad libitum*-fed selected birds took such short steps because the anterior position of their CG results in an unstable equilibrium developing more quickly, necessitating the subsequent foot to be rapidly placed down. Taking shorter steps also decreases the swing phase, when the subject is supported by only one leg, and so further improves stability. At slower speeds, taking shorter steps is more energy efficient, as there is a smaller vertical excursion of the CG each time. The decrease in cadence seen in all groups

with age also decreases the duration of single support, as a decrease in cadence increases the cycle length mainly by increasing the stance phase.

The selected birds demonstrated two further ways of increasing stability. Step width increased significantly as the selected birds grew, in contrast to the relaxed birds. Secondly, the step angle was considerably greater in the *ad libitum*-fed selected birds, which turned their feet outwards (the birds in the other groups all turned their feet inwards). The fact that all the broilers had slightly less inward turning of the foot than Brown Leghorns suggests a degree of instability in all these birds.

Further evidence that the gait of the selected birds (in particular the *ad libitum*-fed birds), is determined by their need for increased stability is reflected in the gait cycle. The birds show much higher % stance and % double contact times than humans, and much lower % swing times. The benefits of long stance and short swing periods are obvious, and the longer double contact times result in a wider support base for a longer period. The longer periods of ground contact also enable the peak forces to be kept lower (for a similar impulse), reducing the stresses on the skeletal system. This is particularly important for the *ad libitum*-fed selected birds, as their shorter legs and heavier bodies result in the muscles having to produce greater forces to move the body, creating higher stresses on the immature bones.

Thus selection has radically altered body conformation, and as a result, the birds have had to dramatically alter they way they walk in order to maintain stability, and keep the forces on the skeleton within safe limits. This leads to the hypothesis that the gait is primarily determined by the conformation of the bird.

Broiler gait was then analysed using the Kistler force plate, to measure GRF's in groups of selected birds raised on different feeding regimes. The hypothesis that pain has a role in the gait of these birds was also tested by comparing the GRF patterns before and after analgesia.

Several significant differences were found between GRF patterns of the broilers in the different feed groups, when the birds were compared at the same age, and the same bodyweight. Although all the broilers moved at speeds in the 'slow' range for Brown Leghorns, both the speed and cadence of the restricted-fed birds were significantly greater than those of the *ad libitum*-fed birds at the same age. On reaching the same bodyweight, the speed of the restricted-fed birds was still significantly greater than the *ad libitum*-fed birds, as a result of greater step lengths, the cadence being similar between the two groups. As

before, the median stance time (over an individual step, and a run) was significantly greater in the in *ad libitum*-fed birds than restricted-fed birds at same age and bodyweight.

As expected from their higher speeds, the peak vertical forces were significantly greater in the restricted (133.93-134.7% bw) than *ad libitum*-fed birds (124.6-126.7% bw), at 6 weeks. Peak vertical force was not always greater than bodyweight over a step, however, which could indicate that the bodyweight is not always fully loaded onto the limb in ground contact, or perhaps the limb sometimes flexes slightly during loading (as in human walking). As the restricted-fed birds grew, however, their peak vertical forces decreased, becoming similar to those of the *ad libitum*-fed birds at the same bodyweight, despite their speed still being greater. This may be because their bodies develop more in proportion, but could also indicate that they develop some gait optimisations.

The braking and propulsive forces were significantly greater (in both size and rate of application) in the restricted than *ad libitum*-fed birds at the same age, which can be explained by the higher speeds of the former birds (expressing the forces as % bw controls for the differences in bodyweight). Comparing the groups at the same bodyweight, however, the differences were less clear. Peak propulsive force decreased with age in the restricted-fed birds, however the braking force did not change, nor did the rate of application of the forces. The braking rate therefore remained significantly greater in the *ad libitum*-fed birds than the restricted-fed birds at the same bodyweight. The relationship between the integrals of the two groups was very variable. As demonstrated in other species, however, the % stance time spent braking or in propulsion was not significantly different between the groups, or speeds.

While there was no significant difference in peak mediolateral force between the two groups at the same age (16.9-20.8% bw), the values were nearly twice those of the Brown Leghorns (10.4-10.9% bw). Surprisingly, at the same bodyweight, the mediolateral forces were significantly greater in the *ad libitum*-fed birds than the restricted fed birds, since by 12 weeks, the forces in the restricted-fed birds had decreased to 12.9-14.6% bw. The large size of the mediolateral forces can be explained by the very wide walking base of the birds, and it is therefore surprising to find that the force decreases as the restricted-fed birds grow. Again, this tends to suggest that the birds develop gait optimisations as they grow (as children do). The X ratios over steps were much higher in the *ad libitum*-fed birds (3-3.5) compared to the restricted-fed birds (2-2.5), indicating that a greater propulsive force is required to 'push' the CG over the stance leg in the *ad libitum*-fed birds.

When analgesic (carprofen) was administered to the birds, three statistically significant changes were found: the speed of the *ad libitum*-fed birds increased after placebo, and the median braking integral and mean % stance time spent in propulsion were

greater in the restricted-fed birds following administration of carprofen. It is unlikely that these changes are of any biological significance, however.

The apparent lack of effect of the analgesic on gait can be interpreted in several ways. While it is possible that the GRF's measured in this experiment do not change with pain, other parameters such as speed of movement do, and might have been expected to change post-analgesia. It is therefore possible that the birds were not in any pain. Equally plausible explanations include: that the drug, dose or route of administration was inappropriate for broilers, that the gait pattern was primarily affected by motivation, or that the gait was primarily determined by conformation (irrespective of the presence of pain). The results presented in this thesis support the latter hypothesis.

Although the results of the analgesia experiment proved inconclusive, an incidental finding was that a high percentage of synovial fluid samples from the broilers contained blood. Old blood (i.e. not due to needle trauma during sampling) was present in 11.11-12.5% of samples from restricted-fed birds, and 56.25% (carprofen) to 62.5 % (placebo), of samples from the *ad libitum*-fed birds. The fact that more than 1 in 2 *ad libitum*-fed birds bled into their joints following relatively minor exercise raises serious welfare concerns. A further surprising finding was the alkalinity of avian synovial fluid, particularly from the *ad libitum*-fed birds (median pH 8.15-8.40), which would have interesting consequences on intra-articular pharmacology.

Because of these findings, a small experiment was carried out to characterise avian synovial fluid. The hock joint was the most accessible, and produced reasonable sample volumes (median 0.4-0.65 ml) compared to species of greater size. Most normal samples were clear or only slightly cloudy, while those from lame birds were markedly turbid, with obvious colour changes, and often lower viscosity. In contrast to the previous broiler experiment, fresh blood was more common than old blood, which was present in only 0-7.5% samples from normal birds, and 18.4% from lame birds. The median SG was found to be higher in normal birds (1.014) than the normal mammal average (1.010), probably due to the presence of fibrin and cells. Examination of synovial membrane samples found mild synovial cell layer thickening and sub-synovial cellular infiltration in most cases, confirming that low grade inflammation is common in broiler hock joints. The alkalinity of synovial fluid from *ad libitum*-fed broilers was also confirmed, median values of pH 7.84-8.0 being established (pH 7.3-7.4 average in mammals).

LAME BIRDS:

Lame birds had much lower speeds and cadences overall, and there were marked differences in the size and duration of the GRF's produced by the sound and lame leg. The birds demonstrated typical antalgic gaits, with long stance times on the sound leg, and very short ones on the lame leg (the swing period of the sound leg was very short, as it was quickly replaced next to lame leg). The double contact periods were therefore surprisingly short, the birds choosing instability over pain. They also demonstrated long periods of one-legged standing, holding the lame leg up. Although this is a normal behaviour in some types of birds, it is not a normal behaviour in broilers.

The vertical loading rate was lower in the lame compared to the sound limb, as was the peak vertical force e.g. as low as 30% bw. Compensatory peaks occurred in the sound limb however, the vertical forces being greater than the normal range. Marked changes also occurred in the craniocaudal forces of the lame leg, periods of one-legged standing producing traces where the initial braking force was separated from the subsequent propulsive force by a prolonged period (when the force fluctuated around zero). The propulsive forces generated by the lame limb were also greater than the braking forces, which can be explained if the bird is very reluctant to place the lame leg down, and decelerates it markedly prior to contacting the ground. This could also explain the greater X ratios seen for the lame leg compared to the sound leg (the greater propulsive force quickly pushing the bodyweight back onto the sound leg).

There were three main ways in which the birds appeared to decrease the load on the painful limb:

1. 'unloading' the lame leg by flapping the wings to raise the CG
2. pushing up on the sound limb to raise the CG, and so delay the time before the lame leg has to decelerate the CG in the subsequent step, and
3. bending the knee and / or hock joint of the lame leg

All of these techniques would then require the sound leg to produce a greater force to reaccelerate the CG upwards again with the next step. Thus the birds appear to use certain human gait optimisations in pathological situations, and add some of their own, such as wing flapping.

The techniques used in this thesis have enabled the gait patterns of normal birds and broilers to be described, quantified and compared. The spatial information obtained using the pedobarograph appears to be most useful for describing normal gait, and differences between the gait patterns of normal birds and broilers. The kinetic studies using the forceplate also produced some interesting results, showing that while the vertical and

craniocaudal forces behaved similarly to those produced in human walking, the mediolateral forces in the birds were much larger. Although the plantar pressure distributions were interesting, it would be difficult to predict how useful these would be in analysing gait.

The results of the gait analysis, combined with the morphometric data, support the hypothesis that the gait of modern selected broilers could be explained by their conformation alone. The hypothesis that pain influences the gait pattern remains unproven, but the presence of pain may be irrelevant from a biomechanical (though not welfare) aspect.

Future work in this field must take into consideration various problems encountered during the research presented in this thesis. The most obvious problem is the difficulty of obtaining runs from the birds without influencing their gait. While it was expected that the birds would not be particularly co-operative, it was underestimated just how much influence motivation could have on their gait, and this must always be considered in interpreting the results. On the technical side, numerous problems were encountered in the development of the pedobarograph. Unfortunately, the initial series of papers (FBD series) gave the impression that developing the system was reasonably straightforward. This was not the case, however, as illustrated by the publication of a follow-up paper (Franks, 1997), nearly 20 years after the original series, specifically to address the numerous problems researchers have encountered. It is unfortunate that these were not discussed more clearly in the original series.

There are many directions in which this work can subsequently be taken however. It would be interesting to analyse and try to characterise gait patterns related to specific pathologies, such as tibial dyschondroplasia. It would also be interesting to measure torque, and to investigate whether there is a link between abnormal torque and the development of bone torsion, for example. If specific abnormalities could be identified, studies could then be undertaken to determine at which stage of growth the parameters start to deviate from normal, and to see if subsequent problems could be predicted from the early gait patterns. This would be particularly useful in selective breeding problems.

From a welfare aspect, further investigation is required to establish whether pain influences the gait of broilers, and this could be done by using a validated analgesic (if one ever becomes available for poultry). Further research is also required to determine the incidence and significance of intra-articular haemorrhage in broilers.

APPENDICES

Appendix 2.1: Ross Broiler Management Manual recommendations

Temperature: from 29 degrees at day-old, gradually decreasing to 21 degrees (thereafter maintained at 20-22 degrees).

Relative humidity: up to 50-60% is acceptable

Lighting range: variable between 0.4 – 22 lux uniform lighting throughout house, usually maintained on 10 lux.

Stocking densities: Ross follow the UK Codes of Recommendation for the Welfare of Livestock (MAFF), which recommend that stocking densities should obtain a biomass of 34.22 kg/m^2. Ross therefore recommends the following stocking densities for broilers:

At 1kg = 34.2 birds / m^2
At 2 kg = 17.1 birds / m^2
At 2.4 kg = 14.3 birds / m^2
At 3.6 kg = 9.5 birds / m^2

For parent stock, the recommendation is that the bird floor space allowance should be increased progressively so that by 28 days birds are stocked at 7-10 birds / m^2

Appendix 2.2: Ross 308 Parent Stock Male Feeding Programme

Age (days)	**0-7**	**8-9**	**10-11**	**12-13**	**14-15**	**16-17**	**18-19**
Feed (g/bird/day)	*ad libitum*	35	37	39	41	43	46
Age (days)	**20-21**	**22-24**	**25-27**	**28-30**	**31-33**	**34-36**	**37-39**
Feed (g/bird/day)	49	51	54	57	60	63	66
Age (days)	**40-42**	**43-45**	**46-49**	**50-56**	**57-63**	**64-77**	
Feed (g/bird/day)	68	70	72	73	74	75	

Appendix 4.1: Roslin Broiler Starter Diet

TMEN	12.4929 MJ/kg
CP	220.1868 g/kg
FAT	54.7894 g/kg
CF	26.31 g/kg
STARCH	411.9 g/kg
DRY	874.007 g/kg
MOISTURE	125.984 g/kg

Appendix 4.2: Bone Ashing Protocol

The ashing process was carried out as described in Thorp and Waddington (1998): the cortical samples were placed in petroleum spirit for 8 hours to extract the fat, then dried at 100°C for 16 hours, and the weights recorded. They were then ashed at 550°C for 12 hours, cooled and re-weighed. The results are given as % bone ash of dry, fat-free weight. To determine calcium and phosphorus content of the bone ash, 10ml of 6N hydrochloric acid was added to the samples and the solution evaporated on a hotplate. The resulting precipitate was dissolved in 20ml deionised water, heated and filtered through No40 ashless filter paper. Phosphorus was measured using the TRAACS 800 automated colorimetric analytical system: molybdovanade reagent was added to the extracted bone solution, and measured at 420nm. Calcium was measured by atomic absorption spectrophotometry at 422.7nm wavelength (with a lanthanum salt added to eliminate interference from other elements).

Appendix 4.3: Processing of bone and synovial tissue sections for histology.

a) Fixation:

Tissue sections were placed in Histosette cassettes, and put into 10% Buffered Neutral Formalin (10% BNF) immediately after dissection.

BNF is made up as follows: 1800mls distilled water, 200mls 40% formaldehyde solution, 13 grams di-sodium hydrogen phosphate (A), 8 grams sodium di-hydrogen phosphate (B). A and B are dissolved together in the 1800mls of distilled water, then the 200mls of formaldehyde added, and mixed well.

The tissue sections are left in fixative for 1 week prior to decalcification (in the case of bone samples only) or embedding.

b) Decalcification (of bone sections):

The bone sections are removed from BNF and immersed in 10% Goodings and Stewart fluid (4000mls distilled water, 500 mls formaldehyde, 500mls formic acid (90%)). The tissues are radiographed daily to determine the end-point of decalcification, at which time the Goodings and Stewart fluid is drained, and the samples washed overnight in running water. They are then replaced into 10% BNF prior to paraffin processing.

c) Embedding:

Tissue is processed in a programmable tissue processor (Shandon Hypercenter XP). The program is made up of 12 steps, all of which take place in a vacuum; the procedure is standard, and involves passing the tissue through ascending concentrations of alcohol, then xylene. The tissue cassettes are then filled with hot wax (using a Reichert-Jung Tissue embedding centre), which is left to cool, after which the tissue (in the wax) can be removed for cutting. A Leitz Wetzeler rotary microtome was used to cut 4μm tissue sections, with a Feather microtome blade (type S35). The trimmed sections were floated in a waterbath (at 50°C), and then lifted onto a polylysine glass slide (BDH-merck, Glasgow). Excess water is removed from the slide, and it is dried overnight in an oven set at 60°C.

d) Staining:

The sections were stained with Haematoxylin and Eosin as follows:

a) sections were deparaffinised and hydrated

b) stained in haemalum for 5 minutes:

- solution A (0.03% sodium iodate, 8% aluminium potassium sulphate, 8% chloral hydrate in distilled water) is heated gently and mixed with
- solution B (0.25% haematoxylin in 9% ethanol) at a ratio of 1.66 : 1
- glacial acetic acid (0.096%) is added after 24 hours

b) sections are then rinsed in water for 5 minutes

c) stained in 2% aqueous eosin Y for 2 minutes

d) dehydrated, cleared and mounted in DPX.

Appendix 5.1: Roslin Layer Diet

TMEN	11.81946 MJ/kg
CP	150.418 g/kg
FAT	70.5392 g/kg
CF	36.323 g/kg
STARCH	401.25 g/kg
DRY	831.982 g/kg
MOISTURE	118.009 g/kg

Appendix 7.1: Staining of synovial fluid smears

The unfixed smears were stained as follows:

a) immersed in May and Grunwald's stain for 6 minutes
b) immersed in a 50 / 50 solution of May and Grunwald's stain and distilled water for 1.5 minutes
c) immersed in a 10% solution of Giemsa stain (pH 6.8, diluted with distilled water) for 15 minutes
d) dipped 5 times in tap water
e) air dried

GLOSSARY

Abduction – movement of a limb or part away from the midline

Acceleration – the rate at which velocity changes (acceleration due to gravity is 9.81 m / $s^{2)}$.

Adduction – movement of a limb or part towards the midline

Amplitude – the voltage level, representing a brightness of a video signal at any given point in time.

Analogue to Digital converter (A/D) – an element of image acquisition that converts an analogue video voltage level sample into a binary quantity.

Analogue – any form of transmission of information where the transmitted signals information-bearing characteristic (usually amplitude or frequency) is varied in direct proportion to the intensity of the sound, or brightness etc.

Ataxia – loss of co-ordination, due to disease of the central nervous system

Bit – the fundamental digital quantity, representing either true (1) or false (0).

Bone stiffness – ability of bone to resist bending

Brightness – the value associated with a pixel representing its grey value from black to white.

Cadence – number of steps taken in a given time (usually steps/minute)

Centre of Gravity – the point at which a single force (of a magnitude equal to the weight of the body or system) should be applied to a rigid body or system to balance exactly the translational and rotational effects of gravitational forces acting on the components of the body or system (i.e. the point at which the weight of the body or system can be considered to act). The centre of gravity will change when the body moves, and can even exist outside of the body e.g. when the body is leaning forward. Thus the 'centre of gravity' refers to the centre of mass in one axis only, that defined by the direction of gravity (i.e. the vertical direction). Note: for all practical purposes, the centre of gravity and the centre of mass are coincident (although in strict physical terms, there is an infinitesimal difference between the two).

Centre of Mass – the point in an assembly of mass particles where the entire mass of the assembly may be regarded as being concentrated and where the resultant of the external forces may be regarded as acting for considerations not concerned with rotation of the assembly.

Centre of Pressure – this is quite independent of the centre of gravity, and is the location of the vertical ground reaction force on a force plate. It is equal and opposite to a weighted average of the location of all downward (action) forces acting on the forceplate.

Contrast – high contrast implies mainly black and white content, medium contrast implies a good spread of grey values, low contrast implies a small spread of greys.

Creep – progressive deformation of soft tissues or materials because of constant loading over an extended period of time.

Cross-talk – forces of different directions influencing each other before collection by the recording system (minimal in modern force plate systems, where each strain gauge is separated).

Digital – representation of a numerical quantity by a number of discrete signals or by the presence or absence of signals in particular positions (see 'bit').

Digitization – the act of sampling and quantifying an analogue video signal

Double Contact Phase – part of cycle where both feet are in contact with the ground

Dynamic range – the spread of grey values in an image

Elastic deformation – a strain in a material that is entirely reversible when the stress is released.

Energy – the capacity to do work, measured in joules. It exists in two basic forms: potential ('stored') energy, equal to work (mass x gravity) x height (i.e. mgh) and kinetic ('movement') energy, equal to ½ mv^2

Floating Phase – period during gait cycle when neither foot is in ground contact (only seen in running or hopping).

Fourier analysis – the determination of the harmonic components of a complex waveform (i.e. the terms of a Fourier series that represents the waveform) either mathematically or by a wave-analyser device.

Force – vector quantity: force applied by normal earth gravity to a mass of 1 kg is 9.81N i.e. 1 N = force applied to give a 1kg mass an acceleration of 1m/ s^2

Frame rate – the rate at which an image is completely updated on the display monitor.

Gait Cycle - the time interval between two successive occurrences of one of the repetitive events of walking. Start of gait cycle is normally taken as point of contact of heel, and in a normal gait, one foot lags behind the other in time by half a cycle.

Grey level – the brightness value assigned to a pixel.

Grey Scale – the discrete grey levels defined in a system e.g. an 8-bit system includes the values from 0 through to 255.

Ground Reaction Forces (GRF's) – the forces that act on the body as a result of its interaction with the ground. Newton's Third Law implies that the GRF's are equal and opposite of those that the body is applying to the ground. Reactive forces are called into play by active forces, but don't in themselves cause a change.

Hysteresis – phenomenon in which a body, when subjected to cyclic loading, exhibits a stress-strain relationship during the loading process different from that in the unloading process.

Image analysis – numerical tabulation of some aspect of an image.

Lateral Rotation – movement that turns the lateral surface of the limb caudomedially

Load – can refer to either mass or weight (the units will distinguish which).

Mass - amount of matter in an object, measured in kilograms

Medial Rotation – movement which turns the lateral surface of the limb craniomedially

Modulus of elasticity – ratio of stress to strain at any point in the elastic region of deformation, yielding a value for stiffness. Units of stress i.e. Pa or N/cm^2

Newton's First Law – 'A body will continue in a state of rest or uniform motion in a straight line unless it is acted upon by an external force'.

Newton's Second Law – 'An external force will cause a body to accelerate in the direction of the force'. The acceleration (a) is equal to the size of the force (F) divided by the mass (m) of the object i.e. a = F/m

Newton's Third Law – 'To every action, there is a reaction, which is equal in magnitude and opposite in direction'.

Nociceptor –receptor which is preferentially sensitive to a noxious or potentially noxious stimulus.

Noise – error present in data that is unrelated to the process being studied (may be random or systematic). Some noise is always present in data collected for biomechanics.

Pixel – the fundamental picture element of a digital image

Plastic deformation – a strain in a material that is permanent, and will not recover when the stress is released.

Pressure – force divided by the area over which the force is applied. Unit is Pascal, equal to $1N/m^2$

Refraction – phenomenon which occurs when a wave crosses a boundary between two media in which its phase velocity differs, leading to a change in the direction of propagation of the waveform.

Refractive Index – the absolute refractive index of a transparent medium is the ratio of the phase velocity of the electromagnetic waves in the free space to that in the medium.

Resolution – the accuracy with which a parameter is divided into discrete levels.

Resolving into components – converting a single force into two or more forces acting in different directions.

Scalar – a quantity which has only magnitude

Smoothing – reducing the effects of noise e.g. by digital or electronic filtering, curve fitting or averaging.

Stance or Support Phase – part of gait cycle where foot is in ground contact

Step – distance between corresponding points on successive footprints of opposite feet (two steps equalling one stride)

Step Angle – used to describe the angle at which a line drawn along the middle toe of each foot would meet.

Strain – deformation that occurs at a point in a structure under loading, either normal strain (change in length) or shear strain (change in angle). Dimensionless (ratio), but often expressed as % deformation (e.g. change in length x 100)

Stress – force per unit area that develops within a structure in response to externally applied loads (can be normal, tensile, compressive or shear). Units: Pascal.

Stride – distance between corresponding points on successive footprints of the same foot.

Stride Time – duration of one complete gait cycle

Swing Phase – part of gait cycle when foot is being advanced through air.

'Toe in' – used to describe the situation where the foot is turned towards the midline of the body (given a positive sign in this thesis).

'Toe out' – used to describe the situation where the foot is turned outwards i.e. away from the midline of the body (given a negative sign in this thesis).

Torque (or moment of force) – the effectiveness of a force to produce rotation about an axis, measured by the product of the force and the perpendicular distance from the line of action of the force to the axis. Units are kg-m, or N-m.

Valgus – angulation of a joint away from midline (i.e. laterally).

Varus – angulation of a joint towards the midline (i.e. medially).

Vector – a quantity with both magnitude and direction

Velocity – vector quantity: the distance covered in a particular direction in a given time (meters /second).

Weight – the force of gravity exerted on an object, measured in Newtons.

Yielding – involves 'plastic flow' within a material as atomic and molecular components of the material are permanently displaced with respect to each other. The stress at which yielding first occurs defines the **'yield strength'**, and the maximal stress reached when failure occurs defines the **failure** (or **fracture**) **strength**.

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Most of the above definitions were taken directly from the following references:

Baxes (1984), Biewener (1992), Rodgers and Cavanagh (1984), and Walker (1988).

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